THE UNIVERSITY OF ALBERTA

STUDIES ON CONTRACTILITY OF THE ISTHMUS OF THE RABBIT FALLOPIAN TUBE

by

GARY WILLIAM HIGGS

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF PHARMACOLOGY

EDMONTON, ALBERTA

Spring, 1972

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled STUDIES ON CONTRACTILITY OF THE ISTHMUS OF THE RABBIT FALLOPIAN TUBE, submitted by GARY WILLIAM HIGGS in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Longitudinal and circular muscle preparations of the ampulla and isthmus of the rabbit fallopian tube under estrogen and progesterone dominance were examined for adrenergic receptors "in vitro", by classical methods. Both α and β receptors were found in all tissues studied and the α receptors were predominant in all cases. A greater amount of β activity was exhibited by the ampulla than the isthmus.

In order to investigate the phenomenon of "tubal locking" of ova, which has been shown to occur in all species, the contractility of the circular muscle of the isthmus was examined quantitatively, with special regard for hormonal status. The spontaneous contractility of estrogen dominant strips was observed as a series of rapid spikes with fairly consistent shape and frequency. Treatment of the animals with progesterone, as well as estrogen, resulted in a modification to the pattern of spontaneous activity. That of progesterone dominant tissues was more irregular in appearance and its area was significantly less than that of tissues under estrogen dominance. The rate of tension increase in spontaneous contractions from progesterone dominant strips was considerably slower than that in contractions from estrogen dominant tissues and the frequency of contraction was affected less by temperature change.

Progesterone treatment also affected the response of the circular muscle of the estrogen dominant isthmus to noradrenaline, shifting the dose-response curve to the right. Modifications in β receptor activity and the catecholamine reuptake process were investigated as possible

explanations for this observation. α receptor deactivation may have been involved.

The area under the curve of the contraction maximum was significantly less in progesterone than in estrogen dominant tissues, whether the stimulant was an adrenergic agent or nonspecific. This last observation was probably a manifestation of some structural change produced by progesterone.

"On the scholar who was made of thought and affection, speech was bestowed. On the researcher who was made of speech, a little thought and affection were bestowed."

Kahlil Gibran

ACKNOWLEDGEMENTS

I am very grateful to my supervisor, Dr. Atef H. Moawad, for the good humor and patience with which he advised and assisted me in the course of this project.

I also wish to thank Mrs. Jan Tapper for her able technical assistance and Dr. Merva Cottle and Mr. Chris Triggle for their help with the fluorescence histochemistry.

This work was financially supported by research grants to Dr. A.H. Moawad from the Canadian Federation for Advancement of Therapeutics and the Medical Research Council of Canada.

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INTRODUCTION

I. General Introduction

Knowledge of the basic physiology of the fallopian tube as well as the influence it exerts on ovum transport is exceedingly sparse. An investigation of some of the factors affecting the contractility of the oviductal musculature might be a reasonable first step in an attempt to fill the gaps which exist. This pharmacological study has been designed, therefore, to determine the relationship between some of the important drugs - especially the autonomics - the female sex hormones, and muscular contractility.

The first reference to something which may have been fallopian tube was made by Aristotle, but he considered it to be part of the uterus. He ascribed no function to it, however, since according to his concept of embryogenesis, the female contribution to the new being, "catamenia" was secreted by the uterus. It was not until the third century B.C. that the human ovaries were detected and described by the Alexandrian anatomist, Herophilus of Chalcedon, and it took Galen to realize that the female "semen" was transported from the ovaries to the uterus by means of the oviduct.

Gabriele Fallopius is given credit for the first accurate description of the fallopian tube which appeared in "Observations Anatomicae Venice" in 1561. Bit by bit knowledge accumulated so that by the middle of the nineteenth century it was realized that fertilization takes place within the oviduct, ciliary and muscular activity are necessary for ovum propulsion and some secretory activity takes place within the oviduct. Henle (1866) was the first to suggest the

possibility of an oviductal sphincter and Assheton (1894) reported that the passage of eggs from the ovarian end of the fallopian tube to the uterus requires 3 days. In a series of papers (1894-96), Langley and Anderson described the source of the sympathetic innervation to the female internal genital organs, including the fallopian tube. Motility studies were soon begun both "in vitro" and "in vivo" using various forms of kymography or tubal insufflation. Kok introduced pharmacological techniques in 1927 when he applied drugs to the oviducts of man and sow "in vitro" and found that in both species different segments of the tube reacted differently to adrenaline. He even took the endocrine phase of the reproductive cycle into account. Exogenous hormonal influence was studied for the first time in 1935 when Burdick and Pincus observed that estrogenic substances could prolong the retention of eggs by the oviducts of rabbits and mice.

Better technology in the past twenty years has lead to more readily acceptable results, yet many controversies and inconsistencies remain. The next sections in the introduction are a review of that part of the most recently determined information which is necessary to serve as a background for the experimental work to follow. Special emphasis has been placed on structural and functional considerations and references to the uterus, a closely related organ, have been included where it was considered appropriate.

II. Structure

A. Embryology

Embryologically, the fallopian tube develops from the same structure as the uterus, that is, the Mullerian duct. The time at which demarcation between the two organs takes place varies with the species (Price, Zaaijer and Ortiz, 1969). In the hamster, mouse and rat which have short gestational periods this separation does not take place until after birth, while the oviduct of the guinea pig has become a separate organ by day 36 which is about half way through the pregnancy.

B. Gross Anatomy

The oviducts of all mammals have many features in common. They can be divided generally into four segments. The thin membranous, funnel-shaped ovarian end is known as the infundibulum. Its actual orientation with respect to the ovary depends on the species. In the rabbit for example, the fluted fimbria at ovulation is in intimate contact with and moving over the surface of the ovary (Blandau and Hayashi, 1966). The next section, abovarian, is the ampulla. Its lumen is wide and its walls are thin. The ampulla represents 50 percent of the total length of the fallopian tube in the rabbit (Harper, 1961; Nilsson and Reinius, 1969) and at least that much in all other mammalian species. Proximally, the ampulla narrows down and meets the isthmus which has a much thicker wall and finer lumen. The segment in which the isthmus joins with the uterus is known as the junctura. The main difference in the appearance of the oviducts among the various species

is the degree of twisting and curling. The tubes of man and the monkey, are relatively straight, while those of the guinea pig, rat and mouse are extremely circuitous (Nilsson and Reinius, 1969). The rabbit fallopian tube lies between these two extremes.

The oviductal wall is composed of three layers. The outermost layer, the tunica serosa, is composed of mesothelium which is continuous with that of peritoneum and connective tissue. The serosal layer is well vascularized. A few smooth muscle fibers are present both subperitoneally and around the vessels.

Lying inside the serosa is the tunica muscularis which is thickest in the isthmus. The mouse and rat have a thin outer longitudinal muscle and a thicker inner circular muscle, while the guinea pig has its circular muscle to the outside of a longitudinal muscle. Rabbits, monkeys and man have three muscle layers (Pauerstein, 1970; Nilsson and Reinius, 1969) a circular layer between an outer and inner longitudinal layer. The outer longitudinal muscle is distributed quite evenly throughout the tube, while the circular muscle is concentrated in the isthmus. Its greatest density is at the ampullary-isthmic junction and it thins out somewhat toward the uterus. The inner longitudinal layer is confined exclusively to the isthmus, is most prominent proximally and reaches its distal limits at the ampullary-isthmic junction. At the junction of the oviduct and uterus, oviductal muscles merge partly with uterine muscles.

The innermost layer of the wall, the mucosal lining, or tunica mucosa, consists of an intricately folded epithelium which has a simple columnar epithelium. Interspersed between the columnar cells are

modified cells of the goblet cell variety which presumably secrete the polysaccharide responsible for the coating of rabbit eggs (Nalbandov, 1969). These nonciliated cells occur with greatest density in the isthmus. The columnar cells are ciliated and appear with greatest frequency in the infundibulum, gradually decreasing in number toward the uterus. Their beat is abovarian. Both the height of the cells and the number of cilia vary with the reproductive state of the female. In castrates the cells become cuboidal and the cilia disappear. According to Borell, Nilsson and Westman (1957) the frequency of the beat of the cilia in the rabbit is increased about 25 percent after ovulation.

The degree and position of mucosal folding, which might reasonably be expected to have an effect on ovum movement varies with the species of interest. In the rabbit, the mucosal membrane of the ampulla forms high branched folds, while those of the isthmus are shorter and thicker. In the junctura mucosal folds terminate in long processes, which project into the uterine lumen.

Kushiya (1968) undertook an electron microscopic study of the muscular coats in the ampulla of the rabbit oviduct with special reference to the neuromuscular relationship. He determined that in this region the population and distribution of smooth muscle cells was similar to that in the vas deferens. He was able to perceive no nexial or protoplasmic connections. According to his observations most of the nerve endings contain granulated vesicles and rarely is there close apposition between nerve endings and smooth muscle cells.

C. Sympathetic Innervation

1. Innervation

Langley and Anderson (1894) reported that the female internal genital organs, including the fallopian tube, are innervated sympathetically by way of the hypogastric nerves. Owman, Rosengren and Sjoberg (1966), by hypogastric nerve section, demonstrated that only approximately 50 percent of the innervation is directly from this source, at least in the rabbit. After denervation, the noradrenaline level in the oviduct decreased to 50 percent of control values. Transection at the utero-vaginal level, or stripping off the vaginal fascia including the rectovaginal septa, revealed a similar reduction in the number of nerves. Total adrenergic denervation was registered when all processes were combined. They, therefore, reasoned that the remaining innervation was by way of "short" adrenergic neurons arising in intramural ganglia. Supporting evidence was produced for this theory through the use of reserpine (Owman and Sjoberg, 1967). A single injection of reserpine 5 mg/kg intravenously lead to the disappearance of half the catecholamine in the oviduct within 4 hours, while 24-30 hours was essential to deplete the other fraction. This result was comparable with previous determinations on the rabbit uterus which is innervated by "short" adrenergic neurons only. Further, fluorescence microscopy showed that generally, it was the vascular innervation which disappeared rapidly, while muscular innervation is by way of "short" adrenergic neurons.

2. Histochemistry

The histochemical fluorescence method developed originally

by Falck to determine the positions of catecholamine containing nerve terminals, has been applied to the fallopian tube of several species - rabbit (Brundin and Wirsen, 1964; Owman and Sjoberg, 1966), human (Brundin and Wirsen, 1964b; Owman, Rosengren and Sjoberg, 1967) rat (Brunden, Fredricsson, Norberg and Swedin, 1969; Norberg and Fredricsson, 1966) - with strikingly similar results. The number of terminals with specific fluorescence increased from the ovarian end of the organ toward the uterus. Few fluorescent fibers occurred in the fimbrial and ampullary segments, and those appeared almost exclusively around blood vessels. In contrast, the rich fluorescence observed in the isthmus was selectively concentrated in the inner circular muscle layer, especially at the junction between the isthmus and ampulla. This is true for rabbit and human tubes, but the densest net of adrenergic nerve terminals in the rat oviduct was at the tubo-uterine junction.

3. Catecholamine Content

a. Normal

Quantitative determinations of noradrenaline levels by spectrofluorimetry have in general confirmed the histochemical findings. Brundin (1964c, 1965) found that the uterine half of the rabbit fallopian tube contained significantly more noradrenaline than the ovarian half - 2.29 vs. 0.34 µg/g. Owman, Rosengren and Sjoberg (1967) found 0.34 ± 0.05 µg/g in the ampulla, 0.49 ± 0.06 µg/g in the isthmus and 0.33 ± 0.03 µg/g in the intramural portion of the human oviduct. In addition, they divided the isthmus into three equal portions, but were able to discern no significant difference in the content among

them. The sheep oviduct was also investigated (Holst, Cox and Braden, 1970). This tube was divided into three equal sections and the results as to noradrenaline contained within these were as follows: ampulla 0.20 µg/g, ampulla-isthmus 0.94 µg/g, isthmus 1.05 µg/g.

b. Hormonal Effects

Preliminary investigations into the effect of ovarian hormones on the catecholamine content in the uterine tube have been carried out. Sjoberg (1967) reported that the weight of the oviduct increased in pregnancy in the rabbit and that there was a corresponding increase in the total level of noradrenaline. Fluorescence revealed no change in the arrangement of sympathetic ground plexus or transmitter content. The number of nerve terminals per amount of smooth muscle remained unchanged. Thus, although the total number of fluorescent nerve terminals increased during pregnancy, their "density" did not vary. In another series of experiments (Sjoberg, 1968) he treated mature estrus rabbits with estradiol for 7 or 14 days and then measured noradrenaline levels fluorimetrically and quantitatively. He observed no increase in fluorescence intensity, but a definite increase in the number of fluorescent terminals. If the noradrenaline content was expressed in µg/pair of tubes then there was a significant increase after estrogen treatment, but if µg/gm were the units used, the result would be unchanged from the untreated case, or slightly decreased. In other words, estrogen treatment produced results, at least quantitatively similar to preganancy. Brundin (1965) treated one group of estrus rabbits with estrogen and another group with estrogen plus progesterone. He found little difference between the noradrenaline contents of the

two groups when the values were in μg/pair of oviducts. No attempt was made to take the tissue weight into account. Falck, Owman, Rosengren and Sjoberg (1969) performed a similar experiment, but they were interested in the rabbit uterus. Estrogen produced a significant increase (approximately 100%) in the total noradrenaline level (μg/pair of horns) after 7 days. Addition of progesterone for a further 7 days reduced the level back to control values. If the weight of the horns was taken into account, both treatments resulted in a decrease in noradrenaline concentration, with the progesterone treated tissues, by far, the lowest of all. The authors explained these results in terms of the type of innervation to the rabbit uterus. The rabbit uterus is innervated by only "short" adrenergic neurons from intramural ganglia while other organs, such as the ovary and the heart, which were used as controls, and which are innervated by "long" neurons were not affected by the hormones. The rabbit oviduct receives one-half of its innervation from intramural ganglia (Owman, Rosengren and Sjoberg, 1966).

4. Adrenergic Receptors

a. Human Fallopian Tube

Rosenblum and Stein (1966) introduced a perfusion pressure catheter into the isthmus of the human oviduct and thus determined the effects of noradrenaline and isopropylnoradrenaline on the circular muscle. Both agents produced a contraction which could be converted to inhibition after use of an α-blocking agent. Isopropyl-noradrenaline became the more effective inhibitor. Isotonic contractions

of the longitudinal muscle were studied using kymography by Nakanishi, Wansbrough and Wood (1967). Perivascular nerve stimulation produced a contraction at an optimal frequency of 3-50 pulses/sec., which could be potentiated by noradrenaline. Both the contraction and the potentiating effect of noradrenaline could be reversed to inhibition by α-blockers. The relaxation could be blocked by propranolol. Later, in another similar study (Nakanishi and Wood, 1968) the previous results were confirmed and β-stimulants (such as isopropylnoradrenaline and adrenaline after phenoxybenzamine) were applied. These led to a decrease in muscle tone. The isthmic region was judged to be more sensitive to autonomic agents and nerve stimulation than the ampulla. The same group has since investigated the effect of various factors on the responses they elicited using noradrenaline and nerve stimulation. Increasing the calcium concentration of their bathing solution to 6.0 mM enhanced both the contractile response to nerve stimulation and exogenous noradrenaline as well as the inhibitory effect of phenoxybenzamine on these. Removal of calcium - down to 0.25 mM - depressed all responses as did increasing the magnesium content of the solutions - 5.5 to 11 mM (Nakanishi et al., 1968(b); 1970). With lowering the bath temperature, there was a gradual decrease in response to stimulation at 30 pulses/sec. Also, as the temperature decreased, the time of onset of contractile response to nerve stimulation and noradrenaline increased.

Setichik, Goldberg, Goldsmith and Pauerstein (1968) applied the perfusion method to the isthmic region in order to monitor the response to noradrenaline. One-third of the tissues investigated exhibited a pressure increase, one-third no change, and one-third

showed a pressure decrease. The majority of these responses could be blocked with the appropriate blocking agent. These results led the authors to suggest that both α and β receptors are present in the circular muscle of the isthmus and that possibly the dominance of the receptor set is controlled by hormonal balance. Coutinho, Maia and Filho (1970) in a preliminary study attempted to take into account the influence of hormones on adrenergic response. "In vivo" recordings were performed using a closed-end pressure transducer, at various times throughout the menstrual cycle. A stimulatory response always followed infusion of noradrenaline or adrenaline and this was followed by a period of inhibition. Less stimulation and more inhibition were observed during the luteal phase of the cycle.

Clearly, based on reports to date, no sound conclusions can be drawn about the types, relative numbers, or function of adrenergic receptors in the human fallopian tube. Hormonal effects are no doubt of the utmost importance and must be taken into account in any future study.

b. Rabbit

Brundin (1964a, 1965) applied an "in vivo" perfusion pressure technique to demonstrate alpha receptors in the circular muscle of the isthmus and ampulla of estrus rabbits. Hypogastric nerve stimulation and the injection of exogenous noradrenaline both produced a pressure increase which could be blocked by phentolamine. The stimulatory response in the isthmus was of greater magnitude than that in the ampulla. Longley, Black and Currie (1968a), using the same technique,

confirmed these results and also showed that the circular muscle of the isthmus contained beta receptors, since its motility was inhibited in response to isopropylnoradrenaline. The inhibition could be blocked by propranolol. The rabbits used had been mated 24 hours previously.

The first study attempting to take into account the influence of ovarian hormones was published by Martin, Ware, Crosby and Pauerstein, 1970. They applied various adrenergic agonists and blocking agents to a longitudinal preparation of isthmus in an organ bath and supplemented this with intraluminal pressure recordings "in vivo". Both normal estrus rabbits and estrus rabbits which had been treated with progesterone (10 mg/day for 2 days preceding the acute experiment) were involved. All preparations exhibited a contraction in response to adrenaline, suggesting the presence of alpha receptors. Beta activity was consistently demonstrated in the progesterone treated animals, while estrus animals demonstrated variable beta activity, at least in the longitudinal preparation. This led to the suggestion that progesterone pretreatment enhances beta receptor activity, but the criteria used for judging enhancement are unclear. A relaxation was defined as a depressant effect on spontaneous contractility, and with estrus rabbits the concentration of isopropylnoradrenaline required to eliminate spontaneous activity was reported, whereas with progesterone-treated animals the concentration needed to reduce activity was given. Coutinho, de Mattos and da Silva (1971) attempted to include hormonal effects on adrenergic stimulation of the rabbit oviduct through "in vivo" pressure recording. The isthmus and ampulla of castrated, estrogen-treated and

estrogen + progesterone-treated animals were examined. Noradrenaline, adrenaline and phenylephrine all produced pressure increases while isopropylnoradrenaline was inhibitory in all animals. These effects could be blocked by the appropriate antagonists. It was observed that estrogen treatment resulted in potentiation of the noradrenaline, adrenaline and phenylephrine responses when compared to those of the castrate. They suggested that progesterone suppressed the alpha receptors since the blocking by phentolamine was more complete, and that it activated the beta receptors because the response to isopropyl-noradrenaline seemed increased. All of these observations were in no way quantitative.

III. Ovum Transport

A. Normal Transport

Many, and often ingenious, have been the methods devised to monitor ovum transport. These have included direct observation (Blandau, 1969), cinematography (Harper, 1961), autoradiography using radioactive artificial ova (Harper, 1964), and the simplest and most often used technique, tubal sectioning followed by flushing (Greenwald, 1961).

The mechanisms by which the infundibulum of the fallopian tube "picks up" the ova have been most extensively studied and reviewed by Blandau (1967,1969). These include: direct action of the cilia of the infundibulum (it is highly ciliated); contractions of the smooth muscle of the mesovarium; rhythmic contractions which change the

relative positions of the oviduct and ovary; and fluid currents set up in the direction of the ostium by cilia activity. He considers the direct ciliary activity to be far the most important.

Since the number of ciliated cells decreases from the ovarian toward the uterine end of the oviduct, no doubt ciliary activity plays a correspondingly less important role in the transport of ova after they leave the infundibulum (Blandau, 1969). Various types of muscular movements have been reported. In the rabbit at least three kinds have been observed (Ichijo, 1960; Harper, 1961,1964; Blandau, 1969): non-conductive segmented, conductive segmented, and peristaltic in both directions, with ampulla toward uterus being the most powerful. The first variety was found mainly in the ampulla, while the latter two predominated in the isthmus. Maia and Coutinho (1970) detected peristalsis and antiperistalsis in the human fallopian tube during the proliferative and luteal phases of the cycle, but only strong peristalsis associated with outbursts of increased activity during menstruation. However, it was not clear from their method of study exactly what was being detected.

Several time studies have been performed on the movement of ova through the fallopian tube. The ampullary portion of the journey has been shown to be rapid in several species: the sow (Burdick, 1942) and the ewe (Wintenberger, 1955); the mouse (Burdick, 1942) and the hamster (Strauss, 1956). Greenwald (1961) estimated the time for the rabbit egg to travel from the infundibulum to the ampullary-isthmic junction at less than two hours. Harper (1966) and Blandau (1969), through direct observation, found the actual time to be considerably

less, 8-10 minutes and 6 minutes 26 seconds, respectively. Although only minutes are required for the ovum to traverse the tubal ampulla of the rabbit, approximately 72 hours after ovulation passes before the blastocyst enters the uterus (Gregory, 1930; Gilchrist, 1932; Black and Asdell, 1959; Greenwald, 1961). Examination of the tube by segments (Greenwald, 1961) revealed that the prolonged delay in ovum transport takes place at the distal entrance to the isthmus. Histologically, no sphincter at this point has ever been detected (Greenwald, 1961) yet there is much indirect evidence that such a functional blockage does exist. Black and Asdell (1958) mounted an oviduct from an estrus rabbit vertically in an organ bath and introduced drops of oil or India ink into the infundibulum in order to watch their migration down the tube. The liquid drops proceeded downward in a segmented manner and stopped 2-3 cm from the uterine end. Black and Asdell (1959) ligated the oviducts of 24 rabbits at the infundibular end a few hours after mating. At intervals the animals were killed and the tubes examined for distention. With one exception, all tubes were distended at 60 hours and periods before. At 72 hours post coitum and later no distention was observed. Brundin (1964a) and Seitchik, Goldberg, Goldsmith and Pauerstein (1968) performed identical experiments on the rabbit and human oviducts, respectively. They recorded simultaneously from pressure transducers placed in the isthmus and the ampulla. Spontaneous activity in either section of the tube was not reflected in the other. Sudden pressure changes introduced into one system by a drug which had no direct effect on the other end, were not detected by the transducer in that

section. Brundin (1965,1968) observed the effects of hypogastric nerve stimulation and noradrenaline on perfusion pressure in the isthmus. He found that a stimulation frequency of 10 pulses/second or noradrenaline perfused at 28μ 1/minute (by way of the ear vein) produced tubal occlusion, since the rate of rise of pressure was the same as when perfusion was against a closed-end catheter.

B. Exogenous Hormones and Ovum Transport

Numerous investigations involving the application of exogenous hormones to determine their effect on the rate of ovum transport have been carried out. Unfortunately, due to the variations in the doses and the time of application a conclusive interpretation is virtually impossible. Black and Asdell (1959) mated several rabbits, ligated the infundibulum of the oviduct, administered estrogen and then examined for distention 100 hours later. If the dose of estrogen was 50 μg daily for four days no distention was observable at 100 hours and the endometrium was progestational in character. When the animals were given a single dose of 1000 μg estrogen followed by 500 μg/day for three days the tube was greatly distended at the time of examination and the endometrium showed estrogen dominance. Progesterone injections (50 or 10 mg) started on the day of mating increased the number of ova recovered from the uterus at 60 hours.

When 25 μg of estradiol cyclopentylpropionate was injected into a rabbit by Greenwald (1961) immediately after mating, increased rate of ovum transport was observed. A dose of 250 μg, however, caused retention of the ova at the ampullary-isthmic junction for as long as

six days. If the animals were ovariectomized and injected with the low dose of estrogen prior to introduction of glass beads (Greenwald, 1968) the transport rate of the tubal contents was not speeded up. Greenwald suggested that possibly the low dose of estrogen worked synergistically with endogenous progesterone. Pauerstein, Fremming and Martin (1970) determined that phenoxybenzamine could antagonize the tubal locking produced by a single injection of 250 μg estradiol.

In 1964, Harper examined transport in ovariectomized rabbits with and without hormone treatment. In the treated cases, estrogen (2.0 μg/day) or progesterone (2.0 μg/day) was injected for four days prior to introduction of artificial eggs. Movement of ova through the untreated ovariectomized animals was more rapid than normal, and progesterone had little effect on this. The estrogen injections, on the other hand, further reduced the time required for the eggs to reach the uterus.

The most careful and complete study of hormonal effects on ovum transport in the rabbit has been carried out by Chang (1966-70). He has applied various types of natural and synthetic estrogens and progestins at different doses and times and has been able to draw several consistent conclusions: progesterone given after ovulation has little effect on tubal transport; estrogen given after ovulation decreases the transport rate in the oviduct, i.e., prolongs tubal locking; progesterone injected before ovulation (usually for three days before) reduces the time the eggs are in the tube, and even though some ova still become fertilized all eventually degenerate. Day and Polge (1968) have reported that also in the pig if progesterone

is introduced long enough before ovulation (36 hours) the eggs will be prematurely expelled into the uterus.

IV. Spontaneous Contractility of the Rabbit Oviduct

Black and Asdell (1958) reported that the spontaneous contractions in the longitudinal muscle of the rabbit oviduct, measured by kymography, consisted of rapid maximal contractions with equally rapid or step by step relaxations. No change was observed in the rate of contraction until four days after ovulation when the intervals between the contractions became longer and the pattern more irregular. Contractions in the ovarian half were lower in amplitude and more irregular than in the uterine half. After 4 days of pseudopregnancy, the tubes were less active (time and amplitude) than if they were from pregnant animals.

In 1959, Black and Asdell opened up fallopian tube rings and mounted them in a bath so that circular muscle activity could be recorded using the kymograph. Low activity was found in the isthmus but it was stronger in the mid-portion and ampulla. Hormonal conditions had no effect. Greenwald (1963) recorded the spontaneous activity in the circular muscle of the isthmus and ampulla "in vivo" using intraluminal pressure transducers. In estrus animals the ampulla showed rapid, weak contractions, irregular in amplitude, while the contractions in the isthmus were much more powerful and uniform, and had a similar rate. One day after ovulation no change could be observed in isthmic contractions, while three days after, the contractions in the isthmus

of all the animals tested had become irregular, slower, and of varying amplitude. Ovulation had little effect on activity in the ampulla. If rabbits were treated with 25 or 250 µg estradiol immediately after ovulation, the estrous contractility pattern remained 3 days after ovulation. Spontaneous activity was recorded in the isthmus 27 days after castration. Three days after injection of the castrate with 25 µg estradiol the normal estrus pattern had returned. If the estrogen-primed castrate was injected daily with 5 mg of progesterone for four days, the isthmic contractions were modified so that they resembled Day 2 or Day 3 of the intact postovulatory oviduct. Brundin (1964b) found spontaneous pressure changes in the ampulla to always be less than 7 mm Hg, while the pressure variation in the isthmus frequently reached 16-50 mm Hg, whether the animals were in estrus or in anestrus.

It is obvious that the information available on this present topic is full of inconsistencies and the reasons are not readily apparent, since important factors concerning methodology have often not been reported. Black and Asdell did not indicate whether or not any tension was applied to their tissues, if they were allowed to incubate and even how many tissues were involved. To measure longitudinal contractions their tissues were mounted V-wise. Although Greenwald used a more sophisticated technique, he also failed to mention such information as the composition of his bathing medium.

V. Drug Effects

Various chemical agents, other than the adrenergics, have been

applied to the fallopian tube to determine their qualitative effect. No parasympathetic innervation has yet been demonstrated in the fallopian tube (Woodruff and Pauerstein, 1969), yet both the circular muscle (Hawkins, 1964) and the longitudinal muscle (Nakanishi, Wansbrough and Wood, 1967) of the human oviduct and the circular muscle of the rabbit tube (Brundin, 1964a; Longley, Black and Currie, 1968) have been shown to contract in response to acetylcholine. Rosenblum and Stein (1966) also found methacholine a stimulant to the circular muscle of the human. Both methacholine and acetylcholine could be blocked by atropine. Brundin (1964a) reported that the ampullary region was more sensitive to a low dose of acetylcholine than the isthmus.

Histamine and barium (Hawkins, 1964 - circular muscle) as well as oxytocin (Rorie and Newton, 1965 - longitudinal muscle; Coutinho and Maia, 1970 - circular muscle) have been shown to have an activating effect on the human oviduct. Vasopressin produced a contraction at low doses, but became inhibitory as the concentration was increased (Coutinho and Maia, 1970). Oxytocin had no effect and vasopressin was inhibitory in the rabbit fallopian tube (Coutinho, de Mattos and da Silva, 1971). Sandberg, Ingelman-Sundberg and Ryden (1963c) determined that papaverine causes a relaxation in all segments of the human tube during all phases of the menstrual cycle.

A fairly extensive investigation into the effects of prostaglandins on the longitudinal muscle of the human tube was carried out by Sandberg, Ingelman-Sundberg and Ryden (1963-65). Prostaglandins E_1 and E_2 exerted a specific action, consisting of contraction of the proximal segment

and relaxation of the rest of the tube. Prostaglandins E_3 and $F_{2\beta}$ were inhibitory to the entire tube, $F_{1\alpha}$, $F_{1\beta}$ and $F_{2\alpha}$ had a stimulatory effect, with the strongest action being exerted by $F_{2\alpha}$. Horton, Main and Thompson (1965) and Brundin (1968) found that an intravenous injection of prostaglandin E_1 into the rabbit resulted in a reduction in perfusion pressure in the isthmic portion of the fallopian tube. This effect was not centrally controlled or secondary to blood pressure changes. It was also demonstrated that the prostaglandin could be absorbed from the vagina.

VI. The Uterus

The uterus has the same embryological origin as the fallopian tube; at the junction of the oviduct and uterus, the oviductal muscles merge partly with uterine muscles (Nilsson and Reinius, 1969) and both organs are subject to control by the same hormones. It should be useful, therefore, to consider briefly studies performed on the uterus concerning its adrenergic activity and contractility and hormonal effects on these.

A. Hormonal Effects on Adrenergic Activity

Marshall (1970) has recently reviewed the literature pertinent to the adrenergic innervation of the uterus. The source of the sympathetic innervation is the same as to the fallopian tube, i.e., the hypogastric nerves. Whether the innervation is direct ("long"

neurons) and/or by way of intramural ganglia ("short neurons) depends on the species. The rabbit, for instance, has only indirect innervation while that to the human is a combination of both types.

The effect produced on the uterus of the rabbit by hypogastric nerve stimulation both "in vitro" and "in situ" seems to depend on the hormonal balance. The virgin untreated and the castrate animal exhibit small contractions. If the castrate is treated with estrogen a contraction which can be blocked by phentolamine results. Treatment with estrogen and progesterone leads to relaxation which is antagonized by propranolol. Stimulation of the human hypogastric nerve "in situ" results in a small contraction followed by relaxation in both follicular and luteal phases of the cycle.

The responses elicited by exogenous catecholamines is similarly modified by hormonal status. "In vitro" and "in vivo" investigation have shown that immature rabbits injected with estrogen, contract in response to adrenaline and noradrenaline. Reversal to relaxation occurs following phenoxybenzamine block. The uteri from animals treated with a progestational agent following estrogen priming relaxed when either adrenaline or noradrenaline was perfused. Propranolol blocked and reversed this response.

B. Myometrial Contractility

The spontaneous contractility of the human uterus throughout the menstrual cycle has been studied in depth by many investigators using several different techniques. By observing the normal cycle plus applying exogenous hormones, the general conclusion has been

reached that the rise and fall in the levels of estrogen and progesterone are the major factors determining the appearance of the spontaneous motility. The rise of progesterone following ovulation is seen to have a generally damping effect on the previously high rapid contractions occurring when only estrogen is present in effective quantities, but the exact nature of the modification to the activity and its precise time of onset are controversial factors.

Various other observations that concern the modification of uterine contractility by hormones have been made. One of the most interesting is the so-called staircase phenomenon. Corner and Csapo (1953) placed uterine strips from the rabbit under isometric tension and stimulated them electrically. When the tissues were from estrogen dominant rabbits the tension declined as the interval between stimuli was lengthened (positive staircase). If progesterone was the dominant hormone the reverse occurred when the interval between pulses increased (negative staircase). This finding has since been confirmed "in vivo" (Schofield, 1955) in the rabbit and in various other species. Schofield also determined that the maximum isometric tension reached in the progesterone dominant tissues was less than in estrogen dominant strips.

VII. Statement of the Problem

It seems reasonably well established that the mammalian ovum in passing through the fallopian tube on its way to the uterus is halted in the region of the ampullary-isthmic junction for approximately three days in all species. The circular muscle is thickest at this point in the oviduct, at least in the monkey, the rabbit and man and

the inner longitudinal muscle reaches its distal limits there. Sympathetic innervation, especially muscular innervation, is richer by far there than in any other region of the organ. Both longitudinal and circular muscles have previously been shown to be responsive to exogenous adrenergic agents as well as sympathetic nerve stimulation. The suggestion has been made that a sympathetically stimulated circular muscle contraction might account for the sphincter-like closing of the isthmus which is probably necessary to permit ovum retardation for the required three day periods.

There are several indications that the progesterone which begins to be produced around the time of ovulation, requires three to four days before its full effect appears. Exogenous progesterone introduced into an animal has been shown not to modify the rate of ovum movement unless that injection takes place a few days before mating. Possibly the delayed action of progesterone is in some way related to the disruption of ovum blockade three days after ovulation. Adrenergic receptor activity and contractility in the uterus have already been demonstrated to be dependent, at least to a degree, on hormonal levels.

Considering the questions so far unanswered this thesis was designed as an attempt to correlate adrenergic activity in the fallopian tube with hormone status. It was decided to use immature rabbits since it is a relatively easy task to direct their hormone balance. Also with the possible exception of monkeys, rabbit fallopian tubes are most like those of the human with respect to muscle type and distribution, as well as adrenergic innervation.

METHODS

I. The Animals

Immature female New Zealand White rabbits weighing 1-2 kilograms were employed in the study. Hormonal treatment was begun by subcutaneously injecting 100 micrograms of the diethylstilbestrol - BDH (dissolved in peanut oil) per day for three or four days. Following this estrogen "priming", the animals were divided into two groups, one to receive progesterone - Nutritional Biochemicals (10 mg per day) plus maintenance dose of diethylstilbestrol (10 micrograms per day) and the other to get only the maintenance doses of estrogen. It was determined by vaginal smear[1] that progesterone became the dominant hormone after four days of progesterone injections. No contractility experiments involving progesterone dominant tissues were performed until at least four days of progesterone treatment had been completed.

The rabbits were sacrificed by means of air embolism. The abdomen was opened and the fallopian tubes were excised, stripped of connective tissue and placed in a normal Krebs Ringer Solution - the ionic contents of which (NaCl 114.32 mM, KCl 5.79 mM, $NaH_2PO_4 \cdot H_2O$ 1.16 mM, Dextrose 49.20 mM, $MgCl_2 \cdot 6H_2O$ 1.16 mM, $CaCl_2 \cdot 2H_2O$ 2.48 mM,

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[1] Normal Krebs Solution was introduced into the vagina by means of an eye dropper. The vaginal walls were scraped with the end of the eye dropper and a sample of the intravaginal fluid was removed and placed on a microscope slide. After the sample had dried it was stained with methylene blue. Under the microscope, many nucleated cells and a large number of glycogen particles were characteristic of progesterone dominance. The nuclei were generally not visible in preparations from an estrogen dominant animals and a smaller number of glycogen granules resulted in a much cleaner appearing smear.

$NaHCO_3$ 21.91 mM - 318 mOsm.) were almost identical to those of rabbit plasma - bubbled with 95% oxygen and 5% carbon dioxide.

II. Contractility Apparatus

In what is to follow the term "isthmus" will represent the 2-3 cm immediately adjacent to the junctura and "ampulla" will refer to the same distance proximal to the infundibulum. The actual length of tissue "hooked" for an experiment was 3-4 mm.

The appropriate piece of tube was placed between two stainless steel hooks - the hooks were attached to the ends of the tissue to record longitudinal contractions and placed through the lumen of "rings" of oviduct when circular muscle was being investigated [it is probable that the circular muscle activity was what was predominately observed in this preparation, but possibly a "spiral" muscle is present (Woodruff and Pauerstein, 1969) in the oviduct which would add a contribution]. The bottom tissue hook was attached to a fixed hook at the base of a 20 ml organ bath and the top one was connected by cotton thread to a strain gauge for recording of isometric (it was possible for small length changes to occur) tension (see Figure 1a). Contractility was monitored using a 4-channel Grass Model 5D polygraph. The organ bath contained Krebs Solution at room temperature (approx. 23°C) and was bubbled with carbagen.

One-half gram of tension was applied to longitudinal strips and one-quarter gram to circular rings. Following the application of tension the tissues were allowed to incubate for at least one hour.

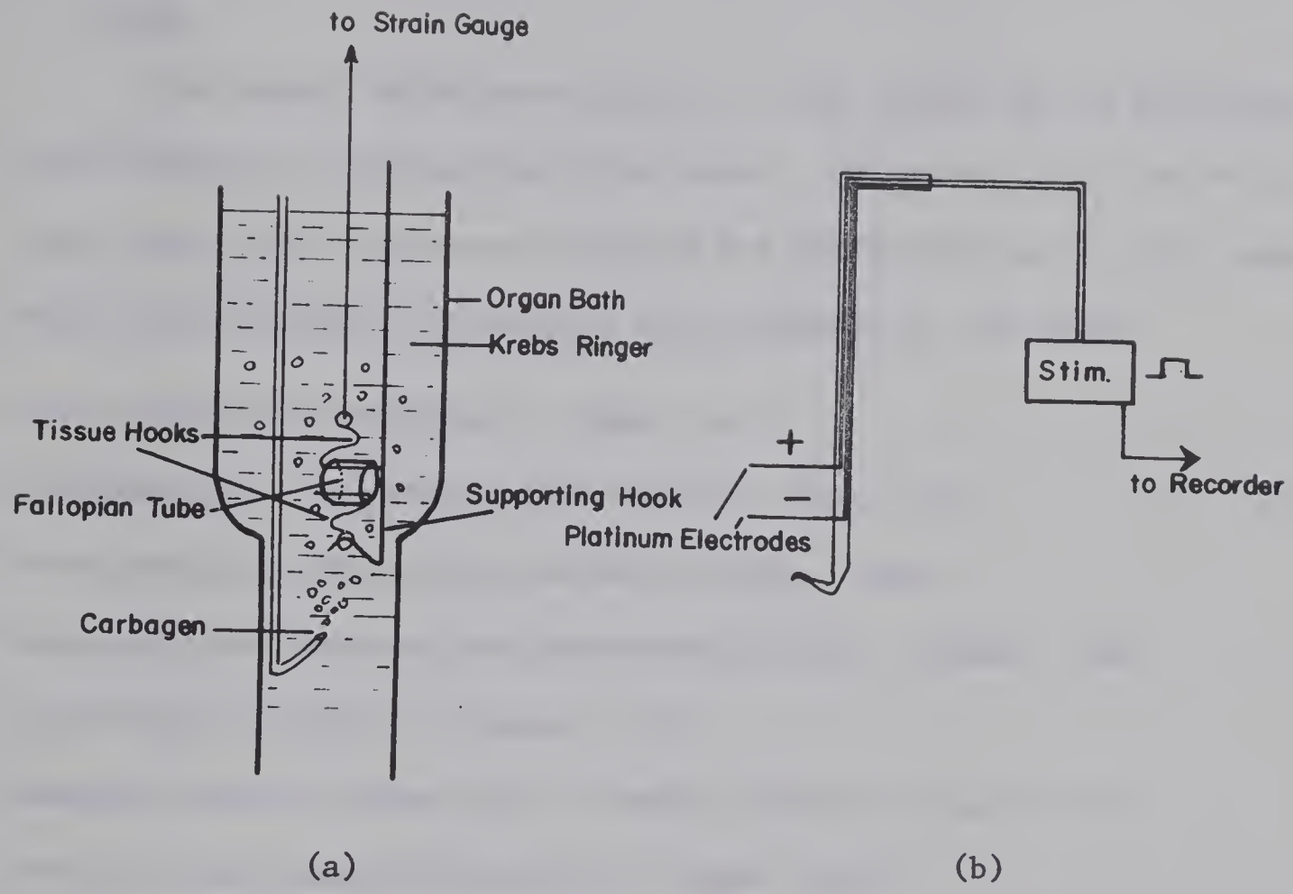

Figure 1 (a) Normal contractility apparatus.

(b) Modification introduced for transmural stimulation.

At some point in this period a priming dose of a stimulant was added to the bath and washed out after only one or two minutes.

III. Solutions

A. Drugs

The agents which were applied to the tissue in the organ bath were dissolved in double-distilled water. Following is a list of the drugs used, where they were obtained and abbreviations of their names which will be applied throughout the remainder of the study:

Adrenaline (L-Epinephrine) - Sigma - A

Noradrenaline (L-Arterenol Bitartrate) - Sigma - NA

Phenylephrine (L-Phenylephrine HCl) - Sigma - Phen.

Isopropylnoradrenaline (DL-Isoproterenol HCl) - Sigma - INA

Propranolol (Ay 6404) - Ayerst - Pl.

Phenoxybenzamine (Dibenyline) - Smith, Kline & French - Pb.

Acetylcholine (Acetylcholine Br) - Sigma - ACh

5-Hydroxytryptamine (Creatinine sulfate complex) - Sigma - 5HT

Oxytocin - Sigma - none

Papaverine HCl - Lilly - Pap.

B. Ca^{++} Concentrations - Response Curve

The determination of the effect of exogenous calcium on contractility first required the removal of tissue Ca^{++} as much as such was possible. Following the normal period of incubation in Krebs solution, a modified, depolarizing medium was introduced into the

organ bath - Ca^{++} = 0, Na^{+} = 50 mM, K^{+} = 55 mM, EDTA* = 1 mM (osmolarity = 246 mOsm). The tissue remained in this solution for $1\frac{1}{2}$ hours and during the latter part of this period no response resulted from the introduction of stimulants such as noradrenaline. At the end of the preparation time, several washings were carried out with the above solution minus EDTA and the experimental procedure was begun.

IV. Transmural Stimulation

A slight modification in the normal contractile apparatus was required to apply transmural stimulation (Figure 1b). A hollow plastic rod 6 mm in diameter was clamped vertically in the tissue bath. A hook was embedded at the bottom of the tube to act as the "fixed hook" as in Figure 1a. Farther up, two platinum wire electrodes were also embedded in such a manner that they would lie one on each side of (but not touching) a tissue which had been mounted for contractility measurements. Leads from the electrodes were passed up through the rod and attached to an AEL Electronic Stimulator, Model 751-B, which was used to deliver monophasic square wave pulses at varying frequencies, durations and voltages. The signal magnet in the stimulator was connected to the signal marker in the Polygraph recorder so that the time-course of the stimulus could be followed automatically. A train of stimuli was applied for a period of 2 minutes and then 5 minutes was allowed to elapse before the next stimulus.

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* Sodium (Tetra) Ethylenediamine Tetraacetate - Fisher

V. 6-Hydroxydopamine Treatment

It has recently been established that 6-hydroxydopamine (6-OHDA) depletes tissues of noradrenaline (NA) (Thoenen, Tranzer and Hausler, 1970; Saner and Thoenen, 1971; Votavova, Boullin and Costa, 1971; De Champlain, 1971) probably by a mechanism of selective uptake into and degeneration of nerve terminals. If this agent were found effective in the fallopian tube then the contribution of the nerve terminals to spontaneous contractility as well as the effect of terminal uptake on exogenous catecholamines could be investigated.

6-OHDA HBr (Regis Chemical Company) was prepared for injection by dissolution at a concentration of 120 mg/ml in a medium containing ascorbic acid (12 mg/ml). Originally, the rabbits were injected intravenously with 60 mg/kg/day for two days, but since some of the animals died, the dose was reduced to 42 mg/kg/day. Contractility experiments were performed 2-4 days following the final injection. Concurrent fluorescence histochemistry was carried out to determine the effectiveness of the treatment.

VI. Fluorescence Histochemistry

A modification of the cryostat method of Derry et al. (1969) based on the original fluorescence technique of Falck (Falck and Owman, 1965) was used to localize catecholamine-containing nerve terminals. The oviducts were removed from the animals, stripped of connective tissue and cut into short segments (approx. 3 mm). To minimize catecholamine diffusion, no longer than 10-15 minutes was allowed

between the time the tissues were excised and when they were attached to mounts, using Cryoform adhesive, in an IEC Cryostat, Model CT1, maintained at -30°C. 5-6μ thick sections of tissue were cut and detached from the microtome blade (ordinary injector razorblade) onto a microscope slide. The slide was then placed in a covered glass container. Usually three slides were prepared from each piece of tissue, two to undergo treatment for development of fluorescence and the third to serve as a heat-treated control (heat develops auto-fluorescence only). Para-formaldehyde (Fisher) was added to the container in which the experimental slides had been placed and the covers of both experimental and control containers were taped closed. The containers were placed in a constant temperature oven at 80°C for one hour to allow the chemical reaction to take place. Following this period the slides were removed from the containers, a drop of paraffin oil placed on each section as the mounting medium and a cover slip applied.

VII. Analysis of Results

Since the tissue under consideration exhibited spontaneous contractility most experimental procedures involved a change in both frequency of spontaneous activity and tension. To allow inclusion of both factors, contractility results were expressed in terms of "area under the curve" measured with a compensating polar planimeter. The area was measured for a five minute period immediately following introduction of the agent.

A. Contraction

The effect of each concentration of an agent producing a stimulatory action was expressed by subtracting from its area, the area under the curve before any drug was added and then dividing the resultant by the 5-minute area under the contraction maximum. The percentage of the maximum area was plotted against concentration of the agent in the bath, on semi-log graph paper.

B. Relaxation

When the tissue relaxed, i.e. the spontaneous contractility declined, upon addition of an agent, the area under the curve before anything was applied was accepted as the maximum and the effect of all concentrations was expressed as a percentage of that value.

C. Statistics

An Olivetti Programma 101 program was used to determine values for mean, standard deviation and standard error of the mean. The unpaired Student's two-tailed t-test was applied when it was necessary to make comparative assessments. 95% was chosen as an acceptable level of significance.

D. Rate of Ascending Segment of a Contractile Response

It was desirable to express the rise to maximum tension of spontaneous contractility and the transmural stimulation in terms of a rate. In each case, several examples of the actual rise were examined and a composite curve of tension vs time plotted. The line joining each pair of points in this composite curve was taken as a

a straight line. The slope was determined for each of the straight lines and plotted against the time representing the midpoint of the line.

RESULTS

I. Spontaneous Contractility

A. Spontaneous Activity at 23°C

522 tissues were involved in the study and 90% of these exhibited spontaneous activity. Only those tissues damaged in handling did not contract spontaneously and these were not used in experimental procedures. Records were scrutinized and compared to determine if any difference in spontaneous activity existed with respect to the nature of the preparation (longitudinal or radial), whether it was from the ampulla or the isthmus, and the hormonal status. These observations were qualitative in the cases of the ampulla and the longitudinal preparation of the isthmus. Quantitative analysis was performed on the circular muscle preparation of the isthmus.

There was no difference in the patterns of spontaneous contractility from a longitudinal and a radial preparation from the same end of the tube and under the same hormonal dominance. Figure 2 expresses well how a change in hormonal dominance affects the spontaneous activity of the circular muscle of the isthmus. The estrogen dominant tissue produced a series of spikes which were much more regular and of greater amplitude than the contractions from the progesterone dominant strip. The right half of the diagram which presents what happened when the chart speed was increased suggests that a contraction coming from a strip of isthmus under estrogen dominance reached a much higher amplitude, more rapidly than did that from a tissue under progesterone control. The return to resting tension was also faster. This last point is more clearly depicted by

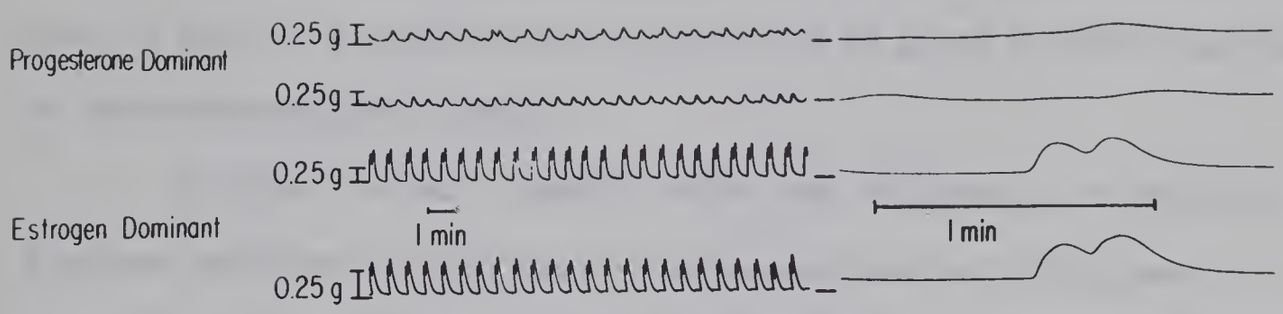


Figure 2. Spontaneous contractility at 23°C.

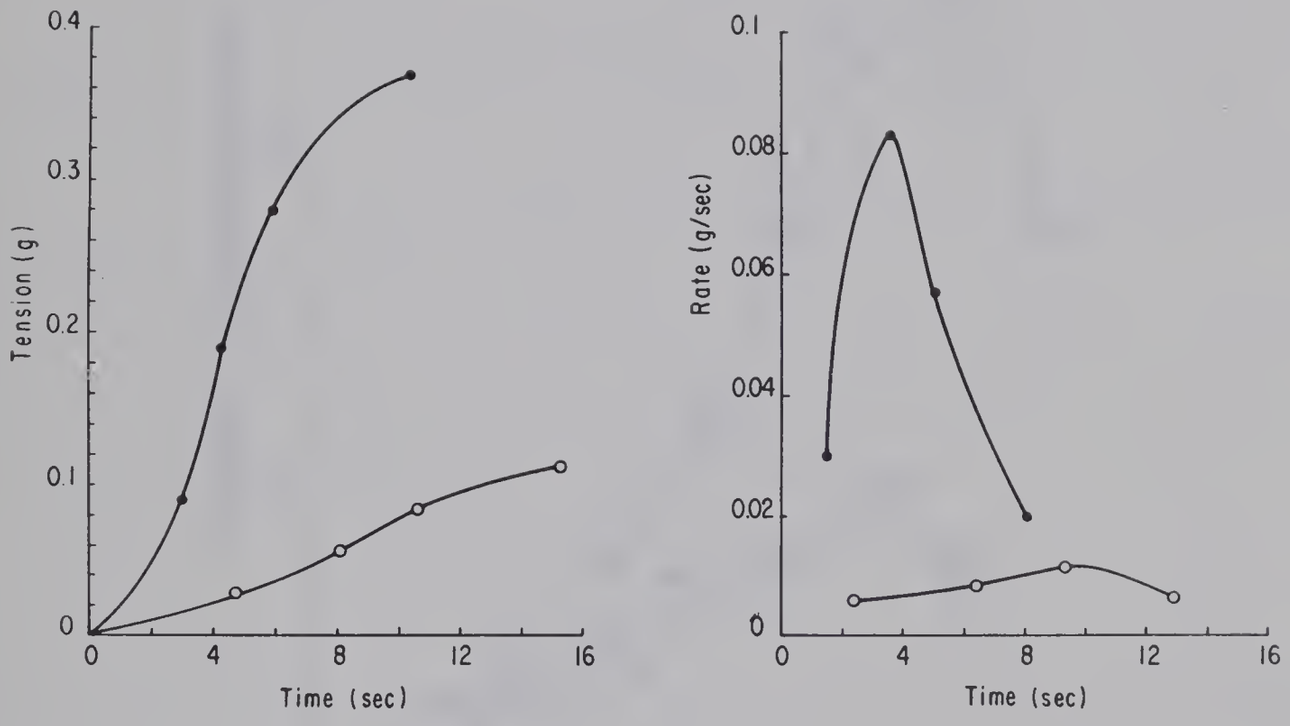
Figure 3, the (a) part of which presents composite spontaneous contractions for estrogen and progesterone dominant tissues. In the (b) section, a rate, determined from the composite contractions, is plotted against time. Both contractions start slowly but the rate for the estrogen dominant strips reaches its maximum much sooner than that for progesterone. At no point in the entire sequence is the rate of rise of a progesterone contraction as great as that representing an estrogen-dominant tissue.

In order to more clearly define the difference in amplitude, 5-minute sections of area under spontaneous contractility were measured and according to Table I, the area under progesterone activity was approximately 42% that under estrogen activity.

In both hormonal states, the ampulla exhibited spontaneous contractility similar to that of the isthmus under progesterone dominance. In other words, the activity in the ampulla was always quite irregular and low in amplitude.

B. Temperature Effects

Figure 4 illustrates the effect produced on the frequency of spontaneous contractility by raising the temperature of the water bath from that normally employed (i.e. room temperature = 23°C) to 37°C. Estrogen dominant spontaneous activity appeared to be much more sensitive to temperature changes. At 23°C no significant difference existed between the frequencies, but as the temperature was increased, the frequency of contraction of estrogen-dominant tissues increased at a more rapid rate than the progesterone-dominant, until



(a) (b)

Figure 3. (a) Composite spontaneous contractions for estrogen -●- and progesterone -o- dominant tissues.

(b) Rate of tension increase of the spontaneous contractions in (a)

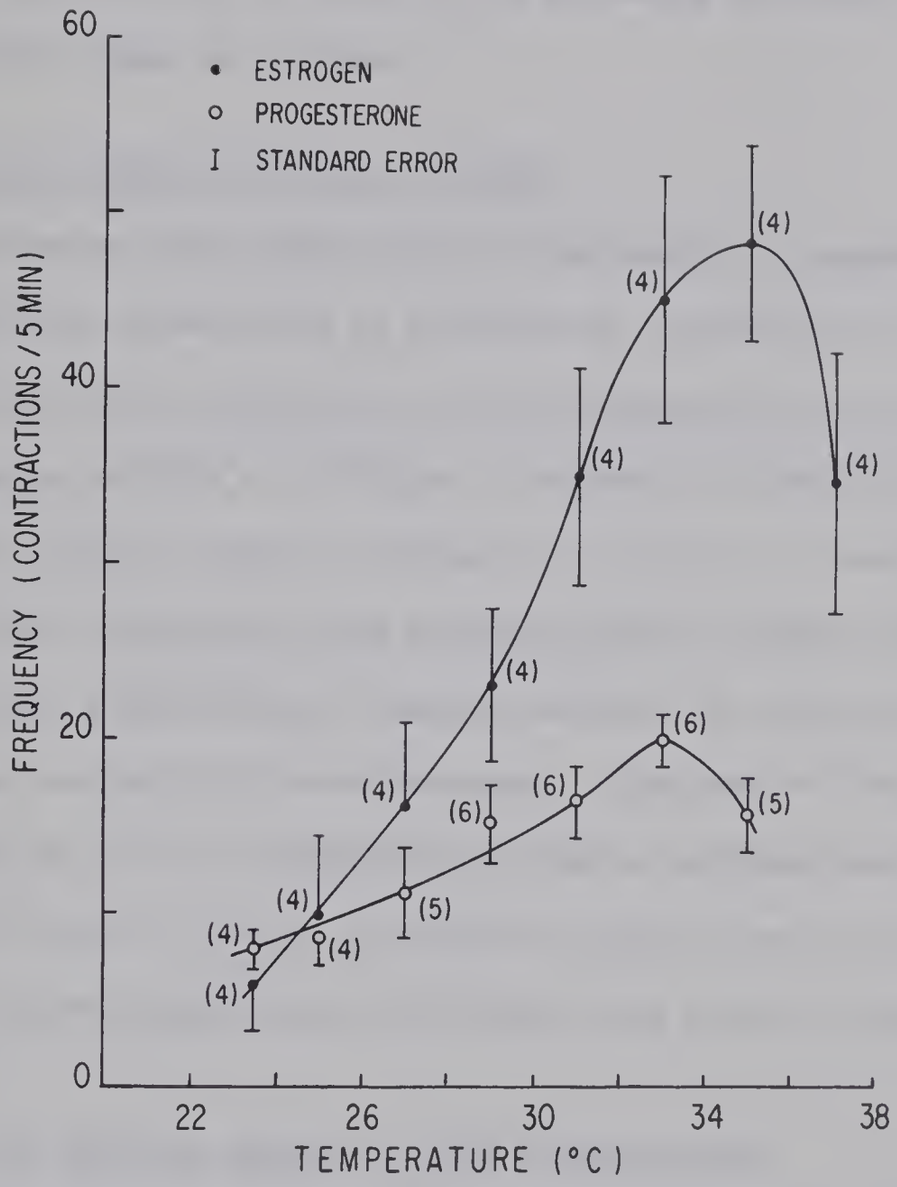


Figure 4. Effect of temperature on the frequency of spontaneous contractility of the circular muscle of the isthmus. The figures in brackets indicate the number of tissues involved in each determination.

at 31°C and beyond, there was a significant difference between the two. The maximum frequency for progesterone contraction was reached at 33°C, and at 35°C for estrogen, and following this the frequencies of contraction began to decline.

C. Effect of 6-Hydroxydopamine (6-OHDA)

Supposing that 6-OHDA acted in the manner as suggested in the methods section, observation of spontaneous contractility following its use should be an indication of the influence of nerve terminals on spontaneous motility. As Figure 5 intimates no qualitative differences could be observed between the activity of control tissues and that from tissues which had received 6-OHDA. Again, in order to introduce some quantitation, 5 minute sections of area under the spontaneous contractility were measured. Reference to Table I will reveal that the ratio of progesterone area to estrogen area was the same as for control tissues, although the actual area in the estrogen dominant case was significantly different from control values.

D. Effect of Blocking Agents in High Concentration

The observation was made that the introduction of large doses of both alpha and beta adrenergic blocking agents into the bath produced an intrinsic effect. Propranolol at 5×10^{-5} mmoles/ml caused a marked depression of spontaneous contractility (i.e. relaxation) after a period of approximately 40 minutes (Figure 6). The intrinsic activity of phenoxybenzamine at 10^{-5} mmoles/ml, as exemplified by Figure 7, appeared after a much shorter incubation from time of introduction, approximately 15 minutes.

Since it became desirable to make use of these antagonists in

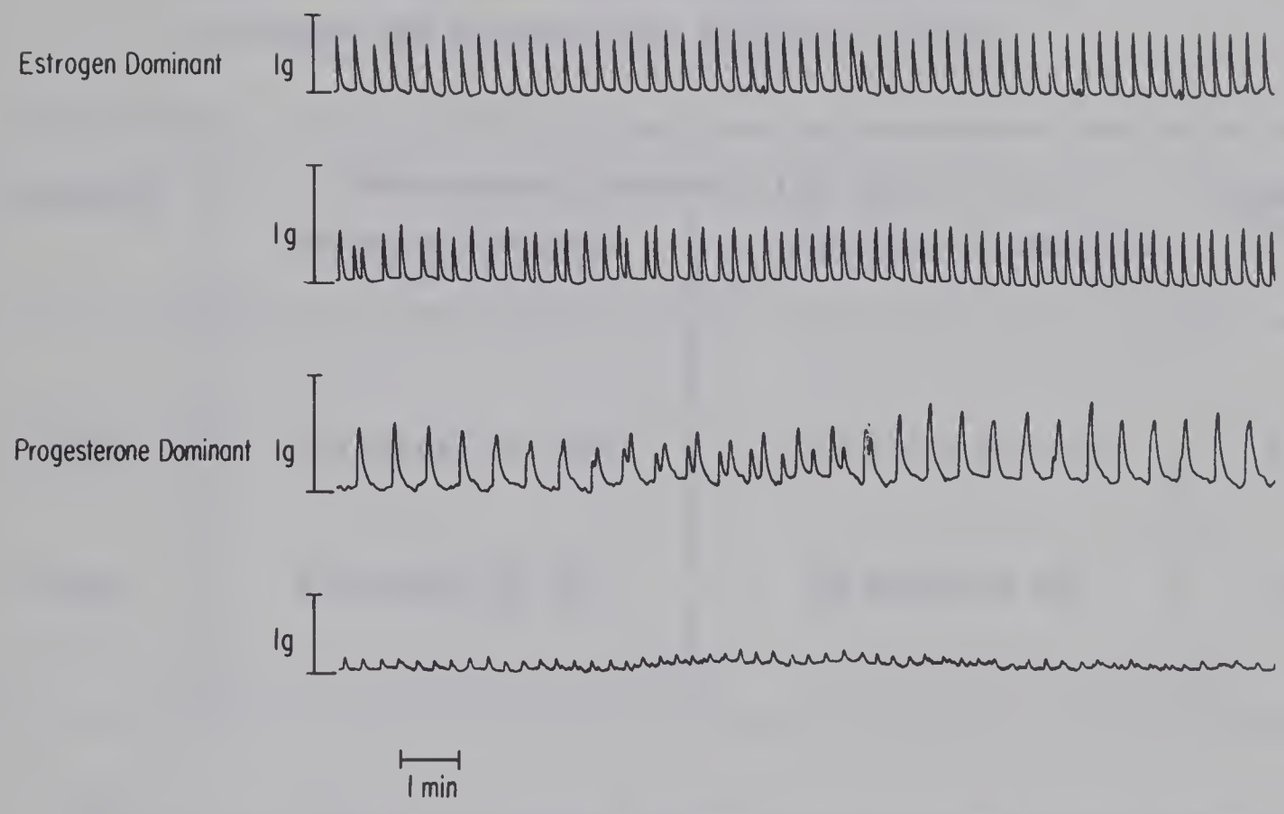


Figure 5. Examples of spontaneous contractility in the circular muscle of the isthmus of fallopian tubes from animals pretreated with 6-OHDA. The bath temperature was 23°C.

Table I

Comparison of Spontaneous Contractility Areas of Estrogen and Progesterone Dominant Tissues

Treatment	Spontaneous Contractility Area ± S.E.M.*		Progesterone / Estrogen
	Estrogen Dominant	Progesterone Dominant	
None	117.86±20.25 (29)†	50.19± 5.24 (31)	0.426
6-OHDA	222.22±33.55 (9)	96.89±25.98 (9)	0.436

*Note significant difference between estrogen and progesterone dominant values.

†Numbers in parentheses indicate number of tissues.

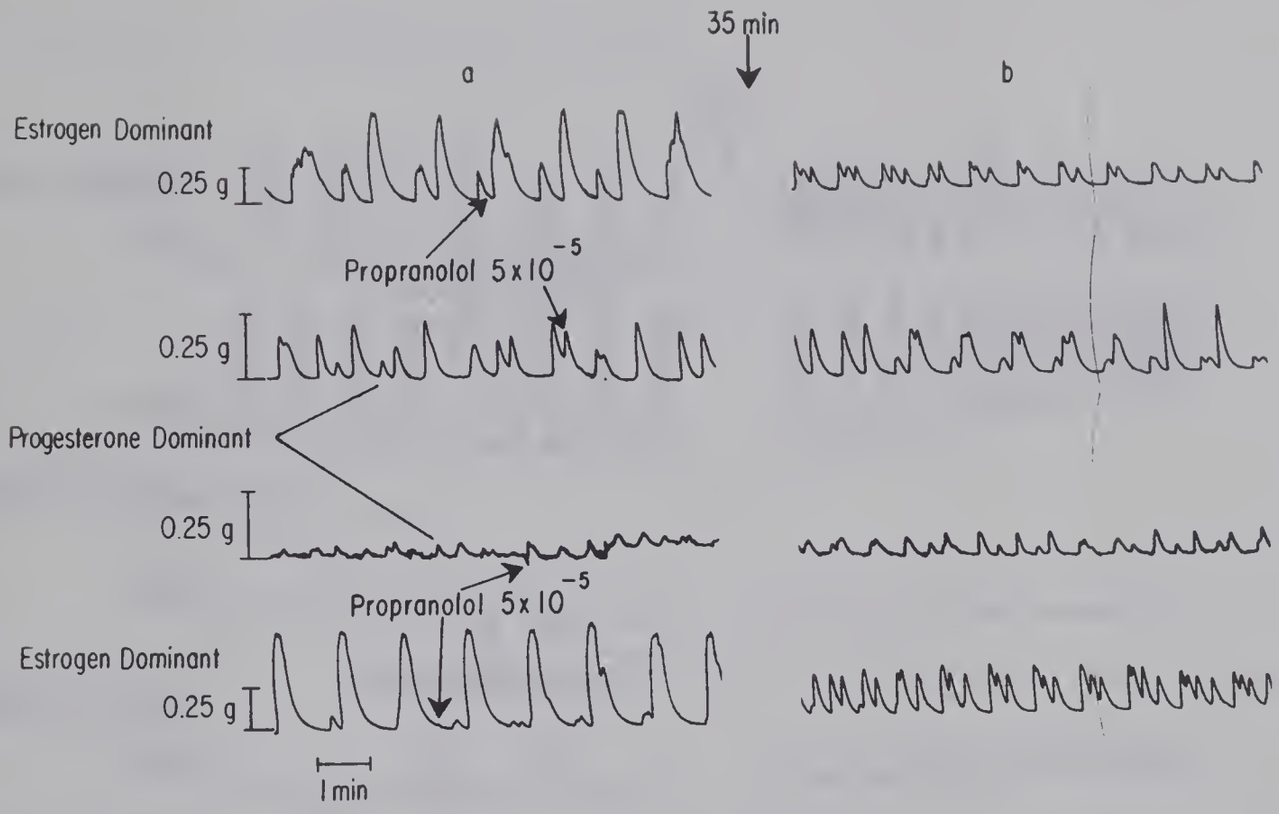


Figure 6. The effect of propranolol at a concentration of 5×10^{-5} mmoles/ml on the circular muscle of the isthmus.

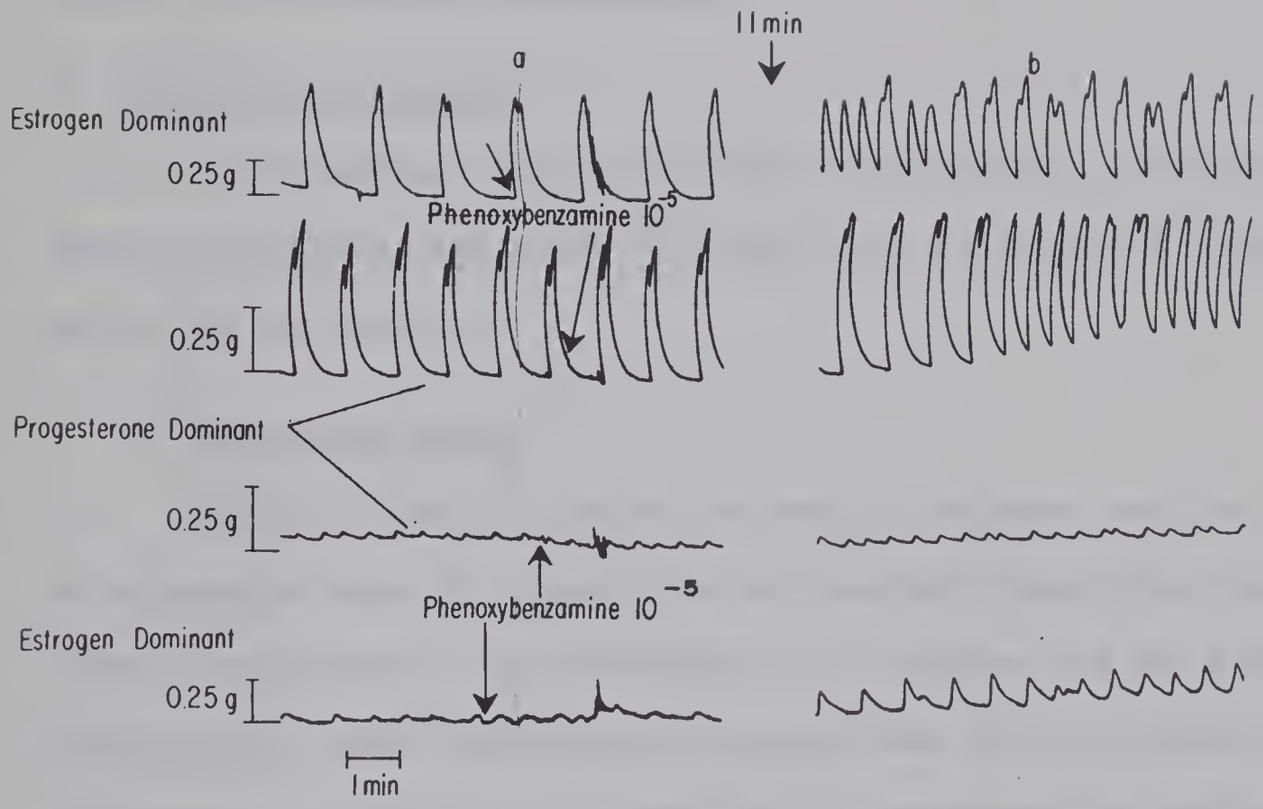


Figure 7. The effect of phenoxybenzamine at a concentration of 10^{-5} mmoles/ml on the circular muscle of the isthmus.

later experiments, it was necessary to find concentrations at which the agents did not possess intrinsic activity yet could still perform their blocking function. These concentrations were 5×10^{-6} mmoles/ml for propranolol and 10^{-6} mmoles/ml for phenoxybenzamine.

II. Response to Stimulants and Relaxants

A. Qualitative Responses

As an initial step in the study of the effects of various agents on contractility in the fallopian tube a qualitative investigation was carried out.

1. Adrenergic Agents

Tables II and III present the results obtained from the addition of adrenergic drugs to tissues from estrogen and progesterone dominant animals respectively. The direction of the response and the effective concentration range (approximate) were the same for the circular and longitudinal preparations. Adrenaline (A), noradrenaline (NA) and phenylephrine (Phen) always resulted in a stimulatory response while isopropylnoradrenaline (INA) produced a depressive effect. A comparison of the effective concentration ranges suggested that the order of potency for stimulation in all cases was adrenaline > noradrenaline > phenylephrine. Larger concentrations of all three agents were necessary to produce a response in the ampulla than were required to contract the isthmus. Complete elimination of spontaneous contractility was brought about by much smaller doses of INA in the ampulla than in the isthmus. The responses to A and NA could be blocked and reversed

Table II

Effect of Adrenergic Agents on Estrogen Dominant Tissues
(circular and longitudinal muscles)

Agent	Effect	Ampulla Effective Conc. Range (mmoles/ml)	Isthmus Effective Conc. Range (mmoles/ml)
Adrenaline	↑	5×10^{-9} - 5×10^{-7}	5×10^{-11} - 10^{-5}
Noradrenaline	↑	5×10^{-8} - 10^{-5}	5×10^{-10} - 2.5×10^{-5}
Phenylephrine	↑	10^{-7} - 10^{-5}	5×10^{-8} - 5×10^{-5}
Isopropyl-noradrenaline	↓	- 5×10^{-9}	2.5×10^{-8} - $\geq 5 \times 10^{-7}$

Table III

Effect of Adrenergic Agents on Progesterone Dominant Tissues
(circular and longitudinal muscles)

Agent	Effect	Ampulla Effective Conc. Range (mmoles/ml)	Isthmus Effective Conc. Range (mmoles/ml)
Adrenaline	↑	5×10^{-10} - 10^{-6}	5×10^{-11} - 2.5×10^{-5}
Noradrenaline	↑	10^{-7} - 5×10^{-6}	5×10^{-10} - 5×10^{-5}
Phenylephrine	↑	10^{-8} - 5×10^{-6}	5×10^{-7} - 5×10^{-5}
Isopropyl-noradrenaline	↓	- 5×10^{-9}	2.5×10^{-8} - $\geq 5 \times 10^{-7}$

to inhibition by phenoxybenzamine (Pb) at 10^{-6} mmoles/ml. The inhibition produced in this manner as well as that due to INA was susceptible to blockage with propranolol (Pl) at 5×10^{-6} mmoles/ml. The contraction resulting from Phen could be eliminated with Pb as well.

2. Other Agents

Several other pharmacological agents were applied to various muscle preparations to monitor their qualitative effects. Tables IV and V summarize the results on estrogen and progesterone-dominant tissues respectively. Acetylcholine (ACh), 5-Hydroxytryptamine (5HT), Ba^{++} and oxytocin were all stimulatory, with the predominant actions of oxytocin and Ba^{++} being to increase the frequency of spontaneous activity. ACh produced its effect over a wide range of concentrations but the ultimate maximum tension reached was never great compared with the maximum amplitude resulting from adrenergic agents like A and NA. High concentration of 5HT had to be present before any response at all could be detected. The effective concentration ranges were very similar for both estrogen and progesterone dominance. There was also little difference between the responses of ampulla and isthmus.

Since the circular muscle of the isthmus was considered to be the muscle of greatest physiological significance in the present study all further quantitative work was performed on it.

B. NA Effect - Quantitative Analysis

1. Animals Untreated (except for hormones)

In order to introduce some quantitative assessment of the NA action a cumulative dose-response curve was prepared (Figure 8). When,

Table IV

Effect of Agents on Estrogen Dominant Tissues

Drug	Muscle	Effect	Effective Conc. Range (mmoles/ml)
Acetylcholine	LA[1]	↑	5×10^{-9} - 10^{-5}
	LI[2]	↑	5×10^{-9} - 10^{-6}
5-hydroxy-tryptamine	LA	↑	2.5×10^{-6} - $>10^{-4}$
	LI	↑	10^{-6} - $>10^{-4}$
Oxytocin	RI[3]	↑ (freq.)	10^{-3} u/ml -
Ba^{++}	RI	↑ (freq.)	0.1 - 0.7 mM

[1]Longitudinal Ampulla

[2]Longitudinal Isthmus

[3]Radial Isthmus

Table V

Effect of Agents on Progesterone Dominant Tissues

Drug	Muscle	Effect	Effective Conc. Range (mmoles/ml)
Acetylcholine	LA[1]	↑	5×10^{-9} - 5×10^{-6}
	LI[2]	↑	10^{-8} - 10^{-5}
5-hydroxy-tryptamine	LA	↑	10^{-6} - $>10^{-4}$
	LI	↑	10^{-6} - $>10^{-4}$
Oxytocin	RI[3]	↑ (freq.)	10^{-3} u/ml-
Ba^{++}	RI	↑	0.1 - 0.7 mM

[1]Longitudinal Ampulla

[2]Longitudinal Isthmus

[3]Radial Isthmus

in plotting % response against concentration, the maximum area under the curve for both estrogen and progesterone-dominant tissues was taken as 100% (solid lines) the progesterone curve was shifted significantly to the right of the estrogen curve. 100% response was never completely obtained on an average, because the tissues reached a maximum contraction over a range of two or three concentrations and often adding more stimulant produced a drop in tension. In addition, a comparison of the maximum areas achieved by progesterone and estrogen dominant tissues revealed a significant difference in that the progesterone maximum was approximately 58% of the estrogen maximum (Table VI). The dashed line in Figure 8 expresses the results for progesterone tissues in terms of the estrogen maximum.

2. Animals previously treated with 6-OHDA

a. Dose-response curve

A second NA dose-response curve was constructed using tissues from animals which had been treated with 6-OHDA, along with a second set of control curves using tissues from untreated animals (Figure 9). This time no significant shift was seen in the controls, due probably to the smaller number of concentrations and tissues employed. However, 6-OHDA treatment produced what appeared to be a significant shift to the left, when either treated tissue was compared with its corresponding control at concentrations of 5×10^{-8} and 5×10^{-7} mmoles/ml. Also a significant difference could be seen between the % maximum response in the two sets of treated tissues at 5×10^{-8} mmoles/ml. A maximum area ratio of 0.48 was found for progesterone/estrogen.

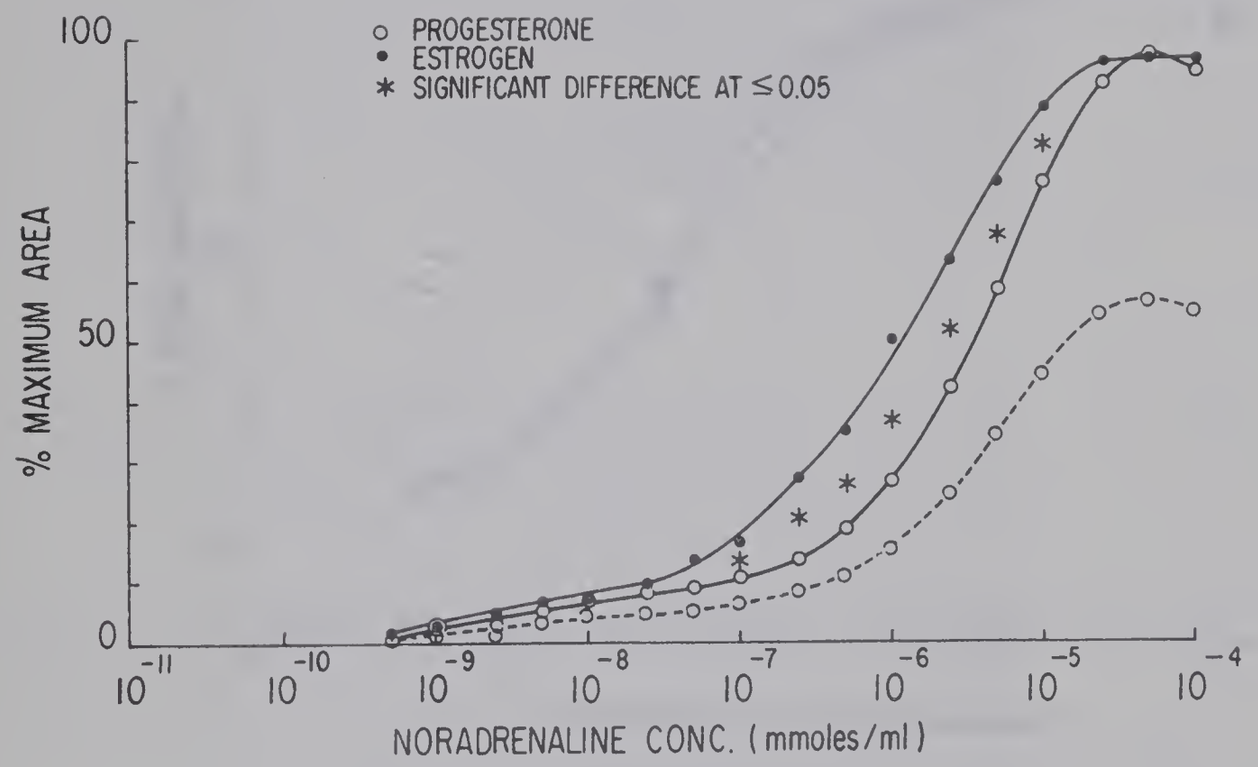


Figure 8. Cumulative dose-response curves representing the response of the circular muscle of the isthmus to noradrenaline. The solid-line (—) curves were constructed by taking the maximum contractility area for each group of tissues as 100%. The dashed-line (---) progesterone curve takes the significant difference between estrogen and progesterone maximal areas into account. 20 estrogen-dominant and 16 progesterone-dominant tissues were involved.

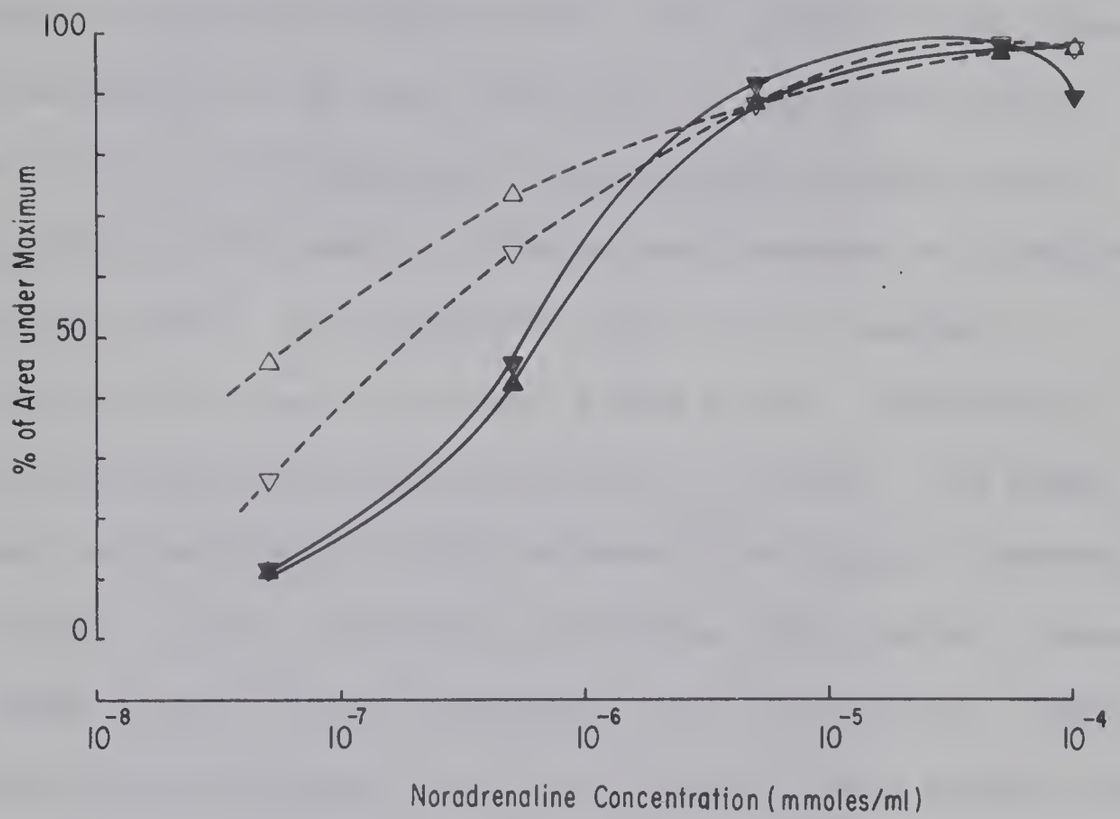


Figure 9. Cumulative dose-response curves representing the response of the circular muscle of the isthmus to noradrenaline. ▲ and ▼, connected by solid lines, stand for estrogen and progesterone dominant controls, respectively. Δ and ∇, connected by dashed lines, represent estrogen and progesterone dominant tissues from animals pretreated with 6-OHDA. 11 control and 9 treated estrogen-dominant tissues and 10 control and 10 treated progesterone-dominant tissues were used.

b. Fluorescence histochemistry

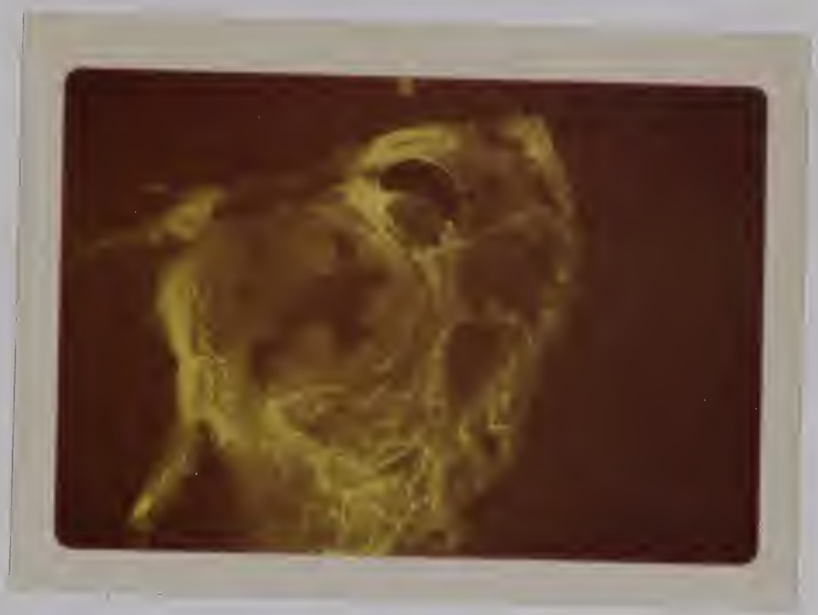
To check the effectiveness of 6-OHDA treatment, the fluorescence method was applied to sections of fallopian tube from animals having had 6-OHDA injections and the results were compared to similar experiments using control animals (Figure 10). The (a) part of the figure presents an example of the rich innervation of the circular muscle layer of a section of isthmus from a progesterone dominant animal. The (b) part shows the result of fluorescence treatment of a similar section after 6-OHDA. No fluorescent fibers can be observed.

As a further test of how well 6-OHDA worked, simultaneous analysis of the heart was carried out by Dr. M. Cottle. The bright fluorescent region in Figure 11(a) represents the muscular innervation of a section of aorta. The tissue in the lower left quadrant, which shows a small amount of autofluorescence only is adventitia. Figure 11(b) reveals how 6-OHDA eliminated the nerve terminals from a similar section.

In the process of examining the innervation in the fallopian tubes from control animals observations were made with respect to nerve terminal density in the various regions of the tube. By far the greatest number of fluorescent terminals existed in the isthmus, especially in the region close to its junction with the ampulla. Innervation was much sparser in the ampulla and infundibulum. These observations were in agreement with what was reported by Brundin (1964d) and Owman and Sjoberg (1966).

C. Phenylephrine

Following the introduction of 5×10^{-6} mmoles of propranolol

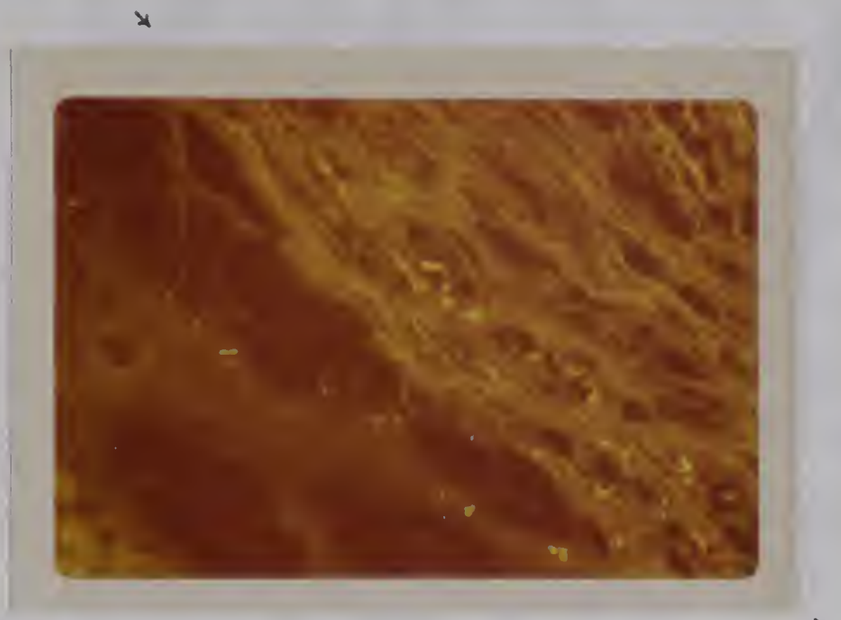


(a)

(b)

Figure 10. (a) An example of the innervation pattern of a section of isthmus from the fallopian tube of a progesterone-dominant rabbit, as determined by fluorescence histochemistry. 39x.

(b) An example from histochemical examination of a similar section from an animal pretreated with 6-OHDA. The background brightness is due to auto-fluorescence.



(a)

(b)

Figure 11. Example of the effect of 6-OHDA on segments of another tissue, aorta.

(a) The bright spots (arrows) are sympathetic endings in aortic muscle.

(b) Similar endings could not be found in aortic tissue (provided through the courtesy of Dr. M. Cottle) from 6-OHDA treated animals.

into the bath a cumulative dose-response relation for phenylephrine was determined. Graphical treatment of the results was similar to that used for NA (Figure 12). There were fewer concentrations at which a significant difference occurred between estrogen and progesterone tissues, at least until the significant difference in maximal areas - progesterone/estrogen ≈ 0.395 (Table VI) - was taken into account. Fewer tissues were involved than in the case of NA.

D. A non-specific Stimulant - Ca^{++}

It was deemed essential that the effect on tubal contractility of a stimulant which did not work on specific "receptors" be established. The experimental procedure employed is described in section III-B of Methods. Figure 13 presents in graphical form, the results which were obtained. Again the treatment of data was similar to that for NA. Significant differences existed between estrogen and progesterone curves at a few points before the difference in maximal area was accounted for - progesterone/estrogen ≈ 0.42 (Table VI).

E. INA

10^{-6} mmoles/ml of phenoxybenzamine was introduced into the bath before dose-response relations were determined for INA. The results of the experiments appear in graphical form in Figure 14. A great deal of variability is suggested by the fact that although the means for the estrogen and progesterone dominant tissues were quite different at all concentrations, a significant difference, at the 95% level, was present at only one concentration. In general, the relaxation was in neither case sufficient to eliminate spontaneous

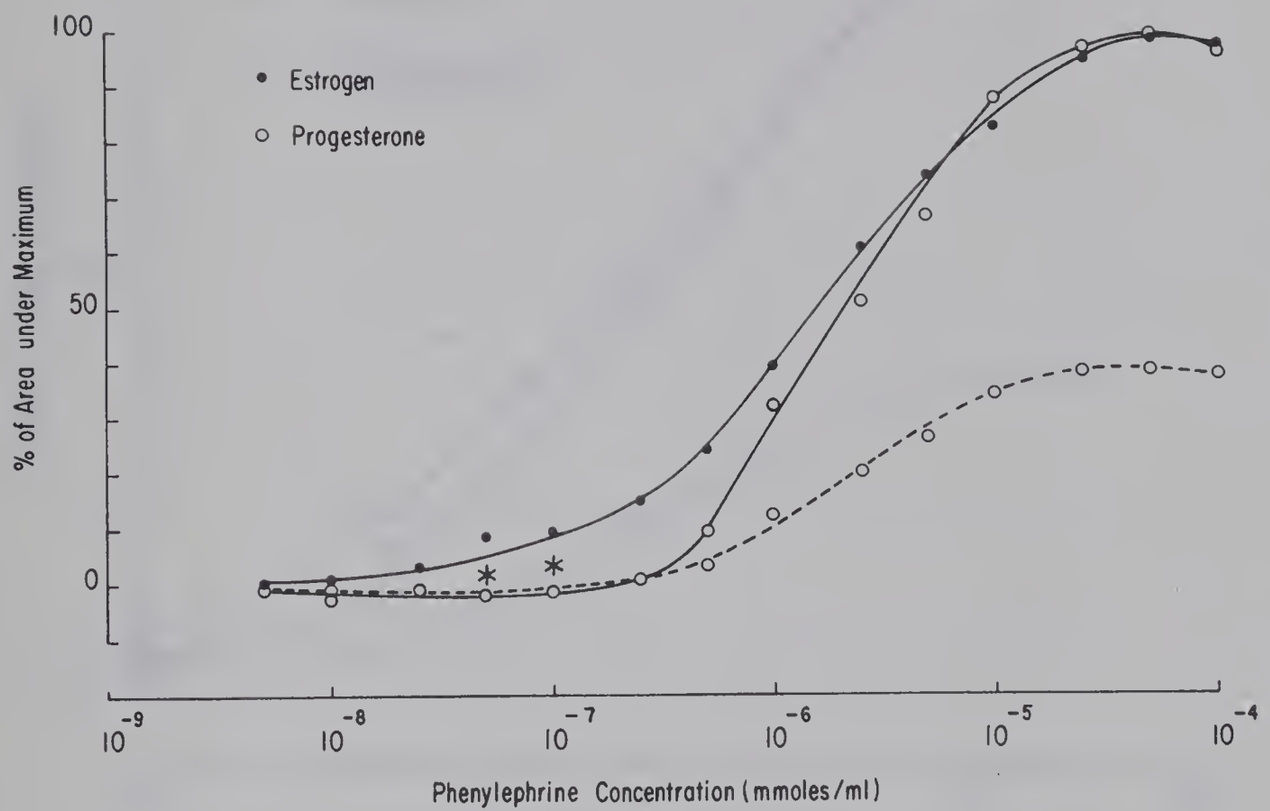


Figure 12. Cumulative dose-response curves representing the response of the circular muscle of the isthmus to phenylephrine after blockade of the β receptors with propranolol 5×10^{-6}. The solid-line (—) curves were constructed by taking the maximum contractility area for each group of tissues as 100%. The dashed-line (---) progesterone curve takes the significant difference between estrogen and progesterone maximal areas into account. 5 estrogen-dominant and 5 progesterone-dominant tissues were involved. * indicates a significant difference.

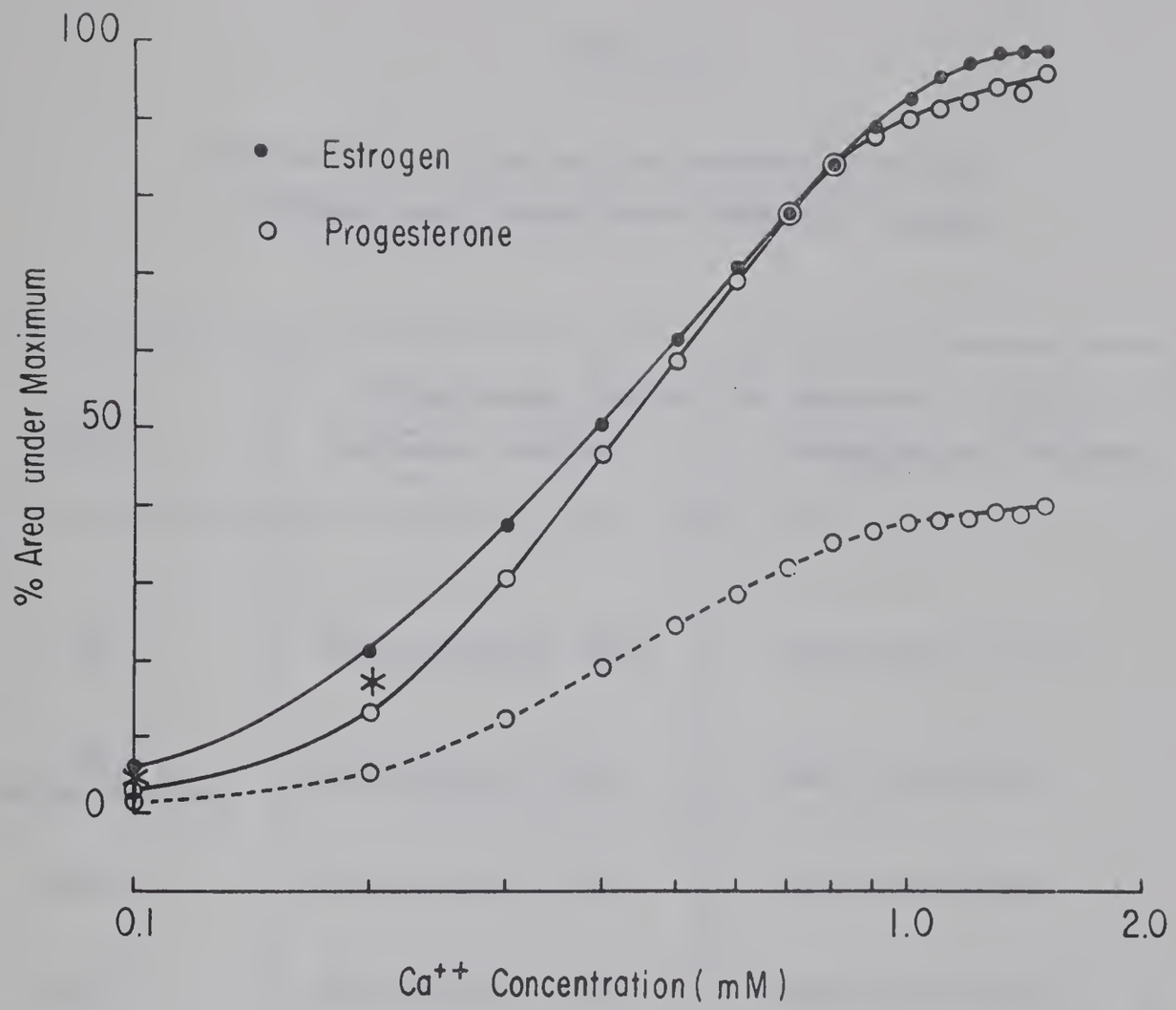


Figure 13. Cumulative dose-response curves representing the response of the circular muscle of the Ca^{++} deficient isthmus to Ca^{++}. The solid-line (—) curves were constructed by taking the maximum contractility area for each group of tissues as 100%. The dashed-line (---) progesterone curve takes the significant difference between estrogen and progesterone maximal areas into account. 15 estrogen-dominant and 15 progesterone-dominant tissues were used. * indicates a significant difference.

Table VI

Comparison of Contraction Maximum Areas for Estrogen and Progesterone Dominant Tissues

Agent	Area under Contraction Maximum ± S.E.M.*		Progesterone / Estrogen
	Estrogen Dominant	Progesterone Dominant	
NA	926.44± 88.87 (16)†	537.35±35.33 (20)	0.580
NA (after 6-OHDA)	1412.89±300.76 (9)	684.11±78.12 (9)	0.484
Phen	1333.20±183.41 (5)	526.80±84.55 (5)	0.395
Ca^{++}	968.87±138.15 (15)	403.33±42.71 (15)	0.416

*Note significant difference between estrogen and progesterone dominant values.

†Numbers in parentheses indicate number of tissues.

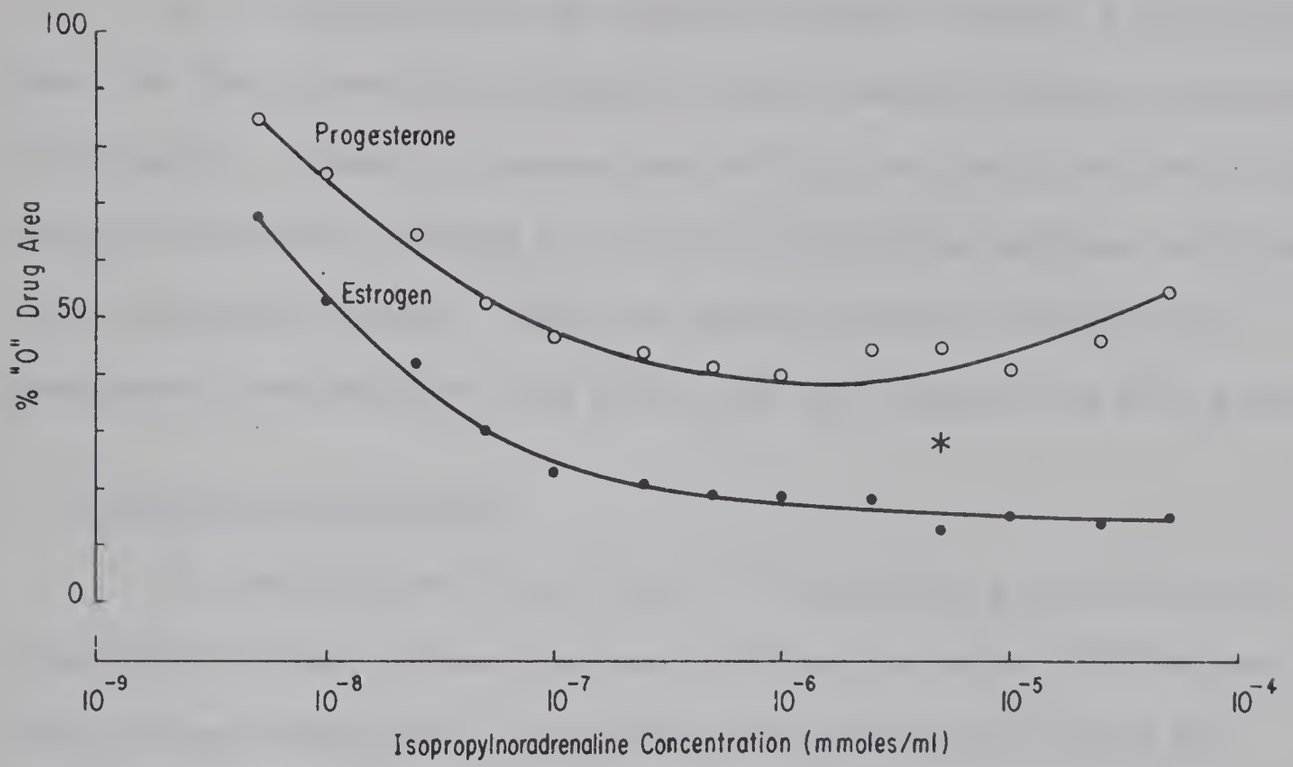


Figure 14. Cumulative dose-response curves representing the response of the circular muscle of the isthmus to isopropylnoradrenaline after blockade of the α receptors with Pb at 10^{-6} mmoles/ml. The spontaneous contractility area before any drug was added was taken as 100%. 12 estrogen-dominant and 11 progesterone-dominant tissues were involved. * indicates a significant difference.

contractility although it seemed more complete in estrogen-dominant tissues.

F. A Non-specific Relaxant - Papaverine

As a comparison for the results obtained with INA a dose-response curve for the supposedly non-specific smooth muscle relaxant, papaverine, was prepared. Figure 15 reveals that at only one point was there a significant difference between the curves representing estrogen and progesterone-dominant tissues. With this agent complete elimination of spontaneous contractility took place with all tissues from both groups.

G. Transmural Stimulation

The application of an electric field across a circular muscle preparation of the isthmus produced a contraction under both hormonal states being investigated. This contraction could be blocked and reversed to inhibition by phenoxybenzamine 10^{-6} mmoles/ml.

Since both voltage and frequency of stimulus could be varied, the conditions for maximum and minimum response were investigated. The first sign of a contractile effect appeared at 8 mAmp, a frequency of 3 pulses per second (pps) and a pulse duration of 1 msec. This observation was more consistent in the estrogen dominant tissue. Often a current of 14-26 mAmp was required before any response could be detected in the progesterone tissue. Over 90% of a maximum response could be produced at 50 mAmp, 3 pps, 1 msec. The maximum was reached at between 70 and 90 mAmp, 3 pps and 1 msec. All quantitative measurements were applied to the maximum response and this was taken as 90 mAmp, 3 pps and 1 msec.

In Table VII it can be seen that the time from the application

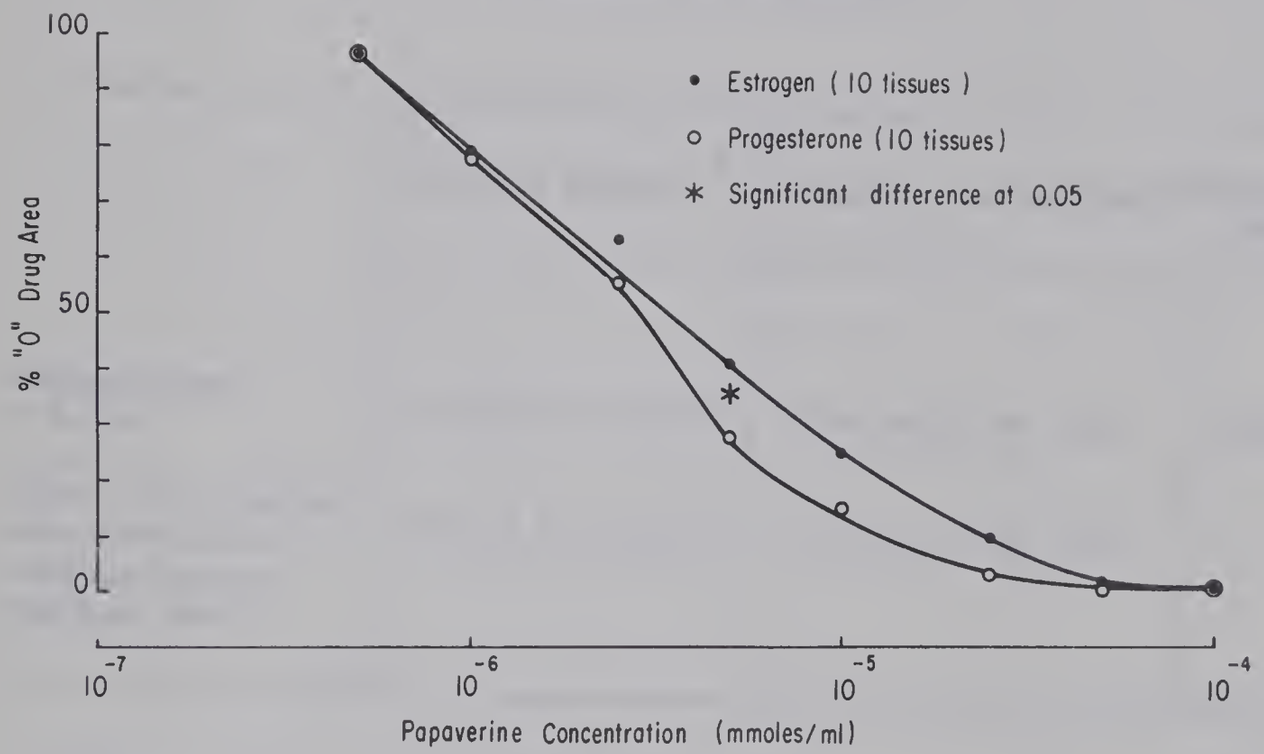


Figure 15. Cumulative dose-response curves representing the response of the circular muscle of the isthmus to papaverine. The spontaneous contractility area before any drug was added was taken as 100%.

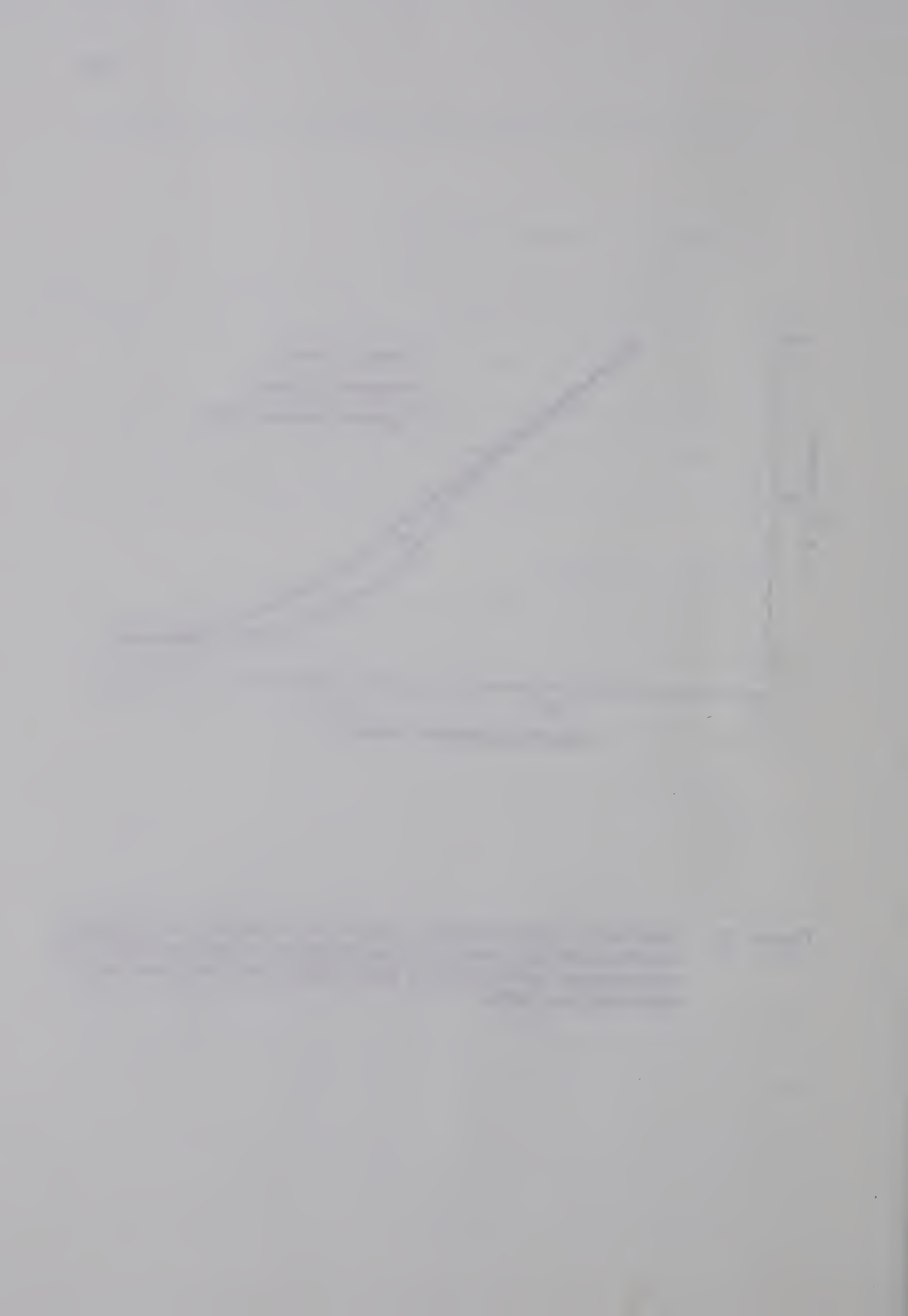


Table VII

Field Stimulation Parameters

	Estrogen Dominant*	Progesterone Dominant*	Progesterone / Estrogen
Maximum Area ± S.E.M.	517.40±44.91 (10)†	264.50±24.58 (10)	0.511
Time from stimulus cessation till resting tension reached (sec.)	78.54± 2.45 (10)	61.11± 2.08 (10)	

*Note significant difference between estrogen and progesterone dominant values.

†Numbers in parentheses indicate number of tissues.

of the stimulus until maximum tension was attained was significantly less for estrogen animals than for progesterone treated ones. In addition, the ratio of progesterone to estrogen maximum areas was 0.51. These two factors are better expressed by the rate curve (Figure 16b). The results from several tissues were anlyzed and from these a typical response to field stimulation was produced (Figure 16a). The rate curves followed treatment of these. A comparison of the estrogen and progesterone rate curves reveals little difference in the rates of rise in the early rapid part of the contraction. Later, however, as the maximum was approached the rate of rise of tension in progesterone tissues became considerably slower than that of estrogen tissues. The actual average tensions reached by the tissues should be noted (Figure 16a).

The tension in progesterone dominant tissues decreased from maximum to resting values significantly faster than that of estrogen tissues (Table VII).

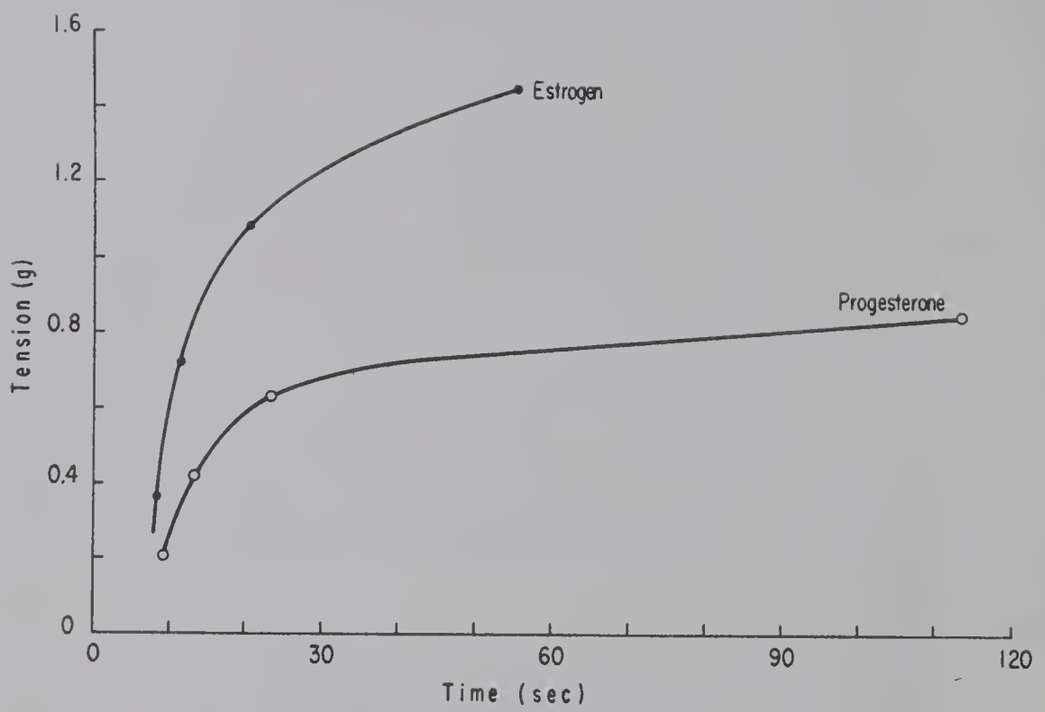


Figure 16 (a). Composite responses of estrogen and progesterone dominant tissues to transmural stimulation.

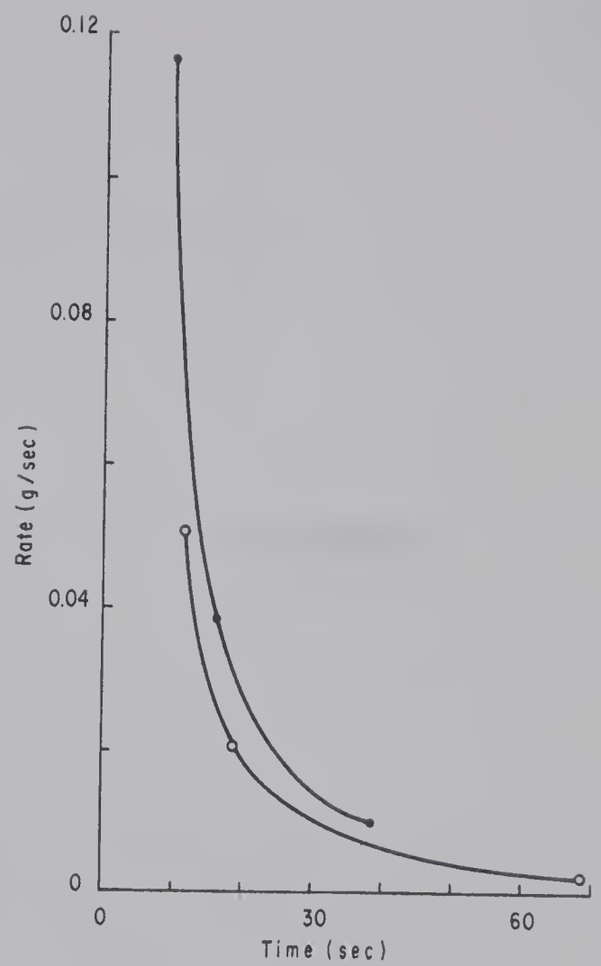


Figure 16 (b). Rate of tension increase of the responses in 16 (a).

DISCUSSION

I. Qualitative Receptor Experiments

The procedure most often used in determining the identity of adrenergic receptors was set out by Levy and Ahlquist (1961). It consists of examining the effects of various adrenergic agonists on the contractility of the muscle in question and then seeing how these are modified by known antagonists. The usual order of potency for agents acting on alpha receptors is A > NA > Phen > INA; and for beta receptors A > INA > NA > Phen. This is true for all muscles so far investigated, except the heart and the gut.

It has previously been demonstrated (see Introduction) that the alpha receptors in the rabbit fallopian tube are excitatory and the beta receptors inhibitory as is the case with all smooth muscles studied to date except the gut. Thus, in the present "in vitro" study the contractile effect produced by A, NA, and Phen, which could be blocked with phenoxybenzamine was accepted as evidence of alpha receptor activity; the inhibition which followed application of INA or A after phenoxybenzamine was taken as an indication of beta activity (Tables II, III). The inhibition was susceptible to blockade by propranolol. Following the established criteria, both alpha and beta receptors were found in the longitudinal and circular muscles of isthmus and ampulla under estrogen and progesterone dominance. These findings agree with what had been determined in previous studies, as far as they went. No one before had demonstrated the presence of β receptors in the circular muscle of the ampulla. In fact, this was the first work to be done on any circular muscle preparation of oviduct

"in vitro".

Under all conditions, the alpha receptor appeared to have dominance over the beta, in number or sensitivity, since adrenaline, which acts equally well on either receptor, always produced a contraction. Smaller concentrations of INA were required to depress the ampulla than the isthmus and larger amounts of alpha agonists were needed to activate it. It is probable, therefore, that the number of beta receptors is greater in the ampulla than the isthmus or the ones present are more sensitive, whereas the number of alpha receptors may be less.

The fact that ACh was capable of stimulation of the tube (Tables IV and V) indicates that cholinergic receptors must be present even though they may not be normally functional, since no parasympathetic innervation of the oviduct has ever been detected. Large concentrations of 5HT and oxytocin were necessary before much effect could be seen. Of all the agents applied the most significant effects, by far, were produced by the adrenergics. One group of chemicals, quite possibly having physiological significance which remains to be investigated, is the prostaglandins.

II. Quantitative Evaluation

An attempt was made in the Introduction to explain why the isthmus of the fallopian tube and especially its circular muscle might be expected to be of significant importance in controlling ovum transport. Because of the reasons stated there, all quantitative experimentation was

restricted to the circular muscle preparation of the isthmus. It should be emphasized that this study was the first to apply quantitative analysis to the investigation of tubal contractility.

The first major observation from the NA dose-response curve which must be dealt with is why progesterone treatment shifted the curve to the right of that representing estrogen tissues when 100% stood for the maximum response of both groups. In other words, why progesterone produced an effect which appeared as if it could be overcome by increasing the dosage of drug. Two of the possible causes which could be invoked to explain this phenomenon are: an influence directly on the receptors, deactivating the alpha receptors and/or activating the beta receptors; a change in the mechanism of reuptake of catecholamines allowing more to be taken up by progesterone-dominant tissues.

The question of catecholamine uptake by nerve terminals was approached through the use of 6-OHDA, which is reported to actually destroy the neurohormone-containing sites (Saner and Thoenen, 1971). Comparison of fluorescence histochemical preparations of both the heart and falllopian tube before and after 6-OHDA treatment suggests that it was quite effective in its task. If the shift to the left of NA dose-response curves after 6-OHDA treatment were interpreted in terms of uptake, it might mean that in the controls part of the effect of exogenous NA, at least the smaller concentrations, was removed by uptake of the drug into nerve terminals. Also, the effect of uptake might be said to be greater in estrogen tissues since the effect produced by 6-OHDA was greater there. On the other hand, the whole effect of 6-OHDA might be explained in terms of a sensitization of

alpha receptors of a deactivation of beta receptors. 6-OHDA is also known to behave at times like a "false transmitter", but that possibility should be minimal here, due to the length of incubation before the NA dose-response curve was begun.

Falck et al. (1969) demonstrated that estrogen treatment of the rabbit uterus increased the total amount of NA, but not the density. Progesterone added, following estrogen priming, reduced the total NA levels back to control levels and the density far below control levels. If the 6-OHDA results were taken as an effect on uptake, then quite possibly the progesterone-dominant oviducts contain fewer nerve terminals than those treated with estrogen only. This fact might have important physiological significance. In any case, the shift in the NA dose-response curve to the right after progesterone is almost certainly not due to enhanced uptake, unless non-specific uptake plays a role.

Beta receptor activity, as influenced by hormones was studied by means of an INA dose-response curve following Pb block of the alpha receptors. A first impression from examining this curve would no doubt be that the progesterone tissues relax less efficiently than the estrogen tissues. Neither group relaxed enough, on the average, for the spontaneous activity to be eliminated. Nevertheless, the process seemed more complete in the estrogen-dominant tissues. This conclusion does not take into account the variability in the results which was so great that a significant difference between estrogen and progesterone curves was registered at only one concentration. Significance at one point is certainly not conclusive evidence that relaxation is more efficient in estrogen tissues or that beta receptor effects are more

potent there. In any event, the conclusion reached by Martin, Ware, Crosby and Pauerstein (1970), i.e. that progesterone pretreatment enhances beta receptor activity "in vivo" was not upheld in this "in vitro" study. However, their work was carried out on mature rabbits and the resulting hormone levels were different from those used here. Also, few experiments were involved and, as was stated in the Introduction, their definition of enhancement of receptor activity is unclear. In conclusion, it seems that beta receptor activation can be ruled out as the cause of the progesterone shift of the NA dose-response curve, if the method of analysis was valid.

The actual ability of the hormonally treated tissues to relax was investigated through the use of a so-called "nonspecific" relaxant of smooth muscle, papaverine. This time, the drug was capable of complete elimination of spontaneous activity. However, on an average, the progesterone tissue reached this state before the estrogen tissue in terms of dose. Although this appears to be opposite to the INA results, again the significant difference was present at one concentration only, which does not allow one to justifiably contend that a real difference in relaxing ability does exist.

A shift, similar to that seen in the NA dose-response curve, appeared in the phenylephrine results, and since these were obtained after β receptor blockade, they offer confirmation to the conclusion that a change in the β receptors is not the factor responsible for progesterone's effect on the NA dose-response curve.

Of these possibilities suggested to explain progesterone's action in shifting the response of the estrogen-dominant tissue to NA

to the right, the only one which remains unshaken is that of deactivation of α receptors. Based on results of the present study, it has not been possible to negate this explanation and therefore it must be accepted as the most plausible so far put forward.

Invocation of the "Occupation Theory" originally proposed by Clark (1933) and modified by Gaddum (1943) allows one to determine the "affinity" of receptors for a drug. The affinity is defined as $1/K_D$, where K_D is a dissociation constant and is equivalent to the concentration of drug producing 50% of a maximal response. Application of these conditions in the present circumstance results in values of 8.3×10^5 ml/mmoles and 2.9×10^5 ml/mmoles as affinities of the adrenergic receptors for NA under estrogen and progesterone dominance, respectively. This indicates that under progesterone, a change in the receptors occurs which leads to reduced affinity for NA. The shift in the NA dose-response curve after progesterone might be interpreted as a form of competitive antagonism. Unfortunately, since the actual concentration of the hormone was not known, Chen and Russel's technique (1950) for determining the affinity of the receptors for the antagonist cannot be applied.

A second observation from the NA dose-response curve which must be explained was why the maximum tension reached by estrogen tissues was much greater than that of progesterone tissues (progesterone/estrogen ≈ 58%), i.e. a noncompetitive effect. This could be explained by a change in the receptors, or it might concern an effect of progesterone on a step or steps in excitation-contraction coupling beyond receptor activation.

An enhancement of β receptor activity by progesterone has already been effectively ruled out, but a depression of α activity has not, and it might be invoked to rationalize the second observation as well as the first. In order to determine whether the inability of the progesterone dominant tissue to contract to as great an extent as the estrogen dominant strip was due to receptor effects, or to some change beyond the receptor level, a dose-response curve with a non-specific stimulant was constructed. Ca^{++} was removed from the tissues and then added back in a depolarizing medium. From the substantial difference in maximum contraction areas it seems reasonable to tentatively rule out changes up to the level of the receptor as being responsible for the action of progesterone in reducing the maximum tension attainable.

Further evidence in support of this contention comes from a consideration of the observations on spontaneous contractility. It was reported that the spontaneous contractility area of progesterone-dominant tissues was considerably less than that of estrogen tissues. In addition, the pattern of contraction was more irregular, the rate of the ascending limb was much slower, and the frequency of contraction was less susceptible to temperature change in the progesterone-dominant oviduct. In other words, progesterone produced a damping effect on the activity much like that observed by Greenwald (1963), who also studied fallopian tubes from pregnant animals and found a similar change to that brought about by addition of exogenous progesterone. As mentioned in the Introduction, an allied effect has been found in the human uterus (Moawad and Bengtsson, 1967, 1968a, 1968b) during the

luteal phase of the menstrual cycle and after therapy with estrogen + gestagen.

Since the sequence of events involved in the propagation of spontaneous contractility is virtually unknown, very little can be said in explanation of the effect of progesterone. Any nervous influence on spontaneous activity was ruled out when it was found that tetrodotoxin could not inhibit such activity (Golenhofen, 1970). The inability of 6-OHDA to modify spontaneous activity in the present study tends to support this view, at least as far as sympathetic innervation is concerned.

Electrical activity can be monitored often, but not always, in conjunction with the spontaneous mechanical activity (Golenhofen, 1970). Since tetrodotoxin, which inhibits changes in sodium permeability, cannot block such occurrences, it has been suggested that the action potentials and subsequently the contraction might be dependent on changes in the permeability of another ion, Ca^{++} (Goodford, 1970). If this were true, the ovarian hormones might be able to exert their influences on contractility through some mechanism involving Ca^{++}.

Indeed, Csapo (1961) proposed that progesterone's effect on the contractility of the rabbit myometrium might be due to an increase in Ca^{++} binding at some cellular site. In the present study it was determined that increasing the external Ca concentration did not eliminate the differences in contractility between estrogen and progesterone tissues. Therefore, if the availability of Ca^{++} is the factor by which the hormones exert their influence, the mechanism of action of progesterone may involve a limitation of the amount of Ca ion reaching

the contractile proteins.

The above interpretation is, of course, extremely speculative; but it might serve as a basis for further investigation. One might propose several other possibilities as solutions to the mechanism of progesterone's action; such as a direct effect on the contractile proteins, etc. Whatever the explanation, it must be able to rationalize the inability of tissues under the influence of progesterone to reach as great a maximum tension as those under the influence of estrogen. Hormone withdrawal effects cannot even yet be excluded as a possible mechanism for what was seen, since all experiments were performed at least 12 hours after the last hormone injection. It must also be remembered that the concentrations of hormone employed were not physiological.

The results from the application of transmural stimulation agree with those observed by applying exogenous agents. The release of catecholamines by sympathetic nerve terminals was able to produce a circular muscle tension increase almost certainly great enough to close off the lumen of the oviduct completely, at least under estrogen control. When progesterone was the dominant hormone, this maximum tension was reduced by approximately one half. The explanations so far put forward for the effects of other agents might equally well apply here. The decreased rate in tension development to transmural stimulation after progesterone and the faster rate of decline of the tension to resting values after removal of the stimulus also fit into the pattern.

The results in this study were deteremined totally "in vitro"

and caution must be used in attempting to transpose them into the physiological situation. A plausable synthesis of observations reported in the present study with those reported in previous investigations of the fallopian tube and ovum transport might be the following: At the time of ovulation, the oviduct is under estrogen dominance and in this state sympathetic discharge is capable of closing off the lumen of the isthmus by contracting the circular muscle. The released ovum rapidly traverses the ampulla but is stopped by the "adrenergic sphincter" in the isthmus. Progesterone concentrations increase after ovulation but 3-4 days is required before its full effect can be felt. Under progesterone dominance the circular muscle of the isthmus is capable of reaching approximately 50% of its previous maximum tension. Thus, when progesterone's influence is felt, the sphincter may be released enough to allow passage of the egg.

III. Conclusions

Keeping in mind experimental conditions, the following conclusions were arrived at:

1. α and β adrenergic receptors are present in the longitudinal and circular muscles of the ampulla and isthmus under estrogen and progesterone dominance. The α receptors are always dominant. There are more β receptors and/or fewer α receptors in the ampulla than in the isthmus.

2. With the method of analysis employed, no difference in β activity could be detected with a change in hormonal status. The α receptor

activity may be modified by progesterone.

3. Spontaneous contractility in the circular muscle of the isthmus is subject to change by hormonal treatment. Under progesterone, the tissue is less susceptible to stimuli. The spontaneous activity is independent of adrenergic nerve influence.

4. Under estrogen, the discharge of catecholamines from adrenergic nerve terminals is capable of contracting the circular muscle of the isthmus to such a degree that the lumen could be drastically reduced in size. The addition of progesterone, somehow modifies the muscle in such a way that it is able to contract only about half as much. This may have important physiological implications.

IV. Future Work

This study has merely scratched the surface of what must be done if the functioning of the oviduct is to be understood with any degree of completeness.

1. Electrical activity has never been monitored in the fallopian tube and this must be investigated before any real insight into the mechanism of action of ovarian hormones can be discovered.

2. If adrenergic innervation plays as large a role in controlling ovum transport as has been intimated, the influence on ovum transport of blocking agents and other drugs affecting sympathetic response should be studied.

3. The prostaglandins may have a physiological role as far as the process of fertilization is concerned and this should be determined.

4. "In vivo" motility studies would be useful in defining the oviduct's function.

5. The prime objective should be to proceed as quickly as possible to the investigation of all facets of the human fallopian tube and animal work should be organized for no other purpose than to hasten this progression. The main reason for this is the possibility of application to population control problems.

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